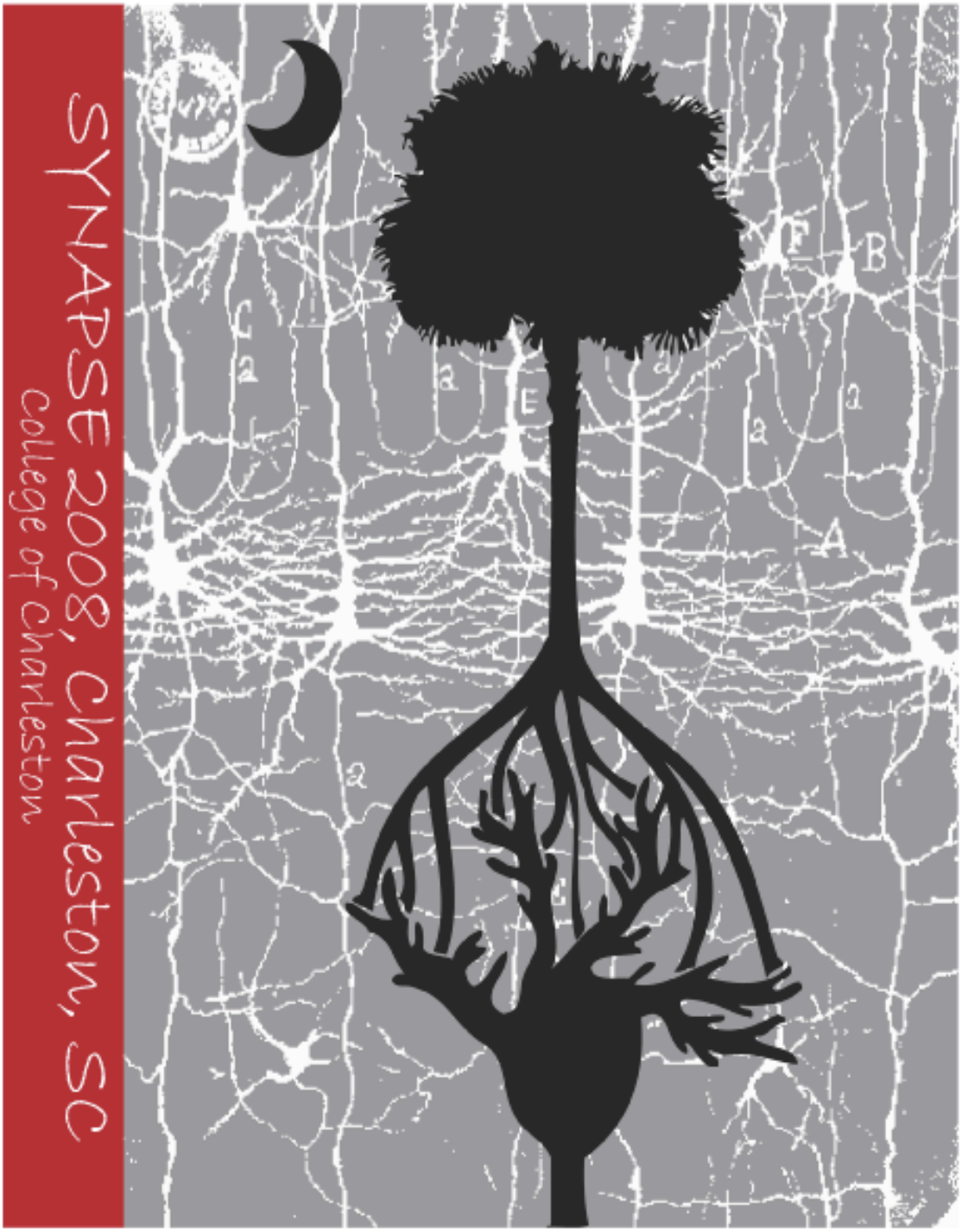


# *Symposium for Young Neuroscientists and Professors of the Southeast*

# Acknowledgements

South Carolina

This symposium was funded by a research symposium grant from the South Carolina EPSCoR/IDeA Program (www.scepscoridea.org).

Support was also provided by the College of Charleston's Undergraduate Research and Creative Activities Office (www.cofc.edu/UR)

We would also like to say a special thanks to Vanessa McNamara in the Office of the Dean of the School of Science and Math for all of her help with scheduling the conference. In addition, we would like to thank Brittany Klein and Barbra Bannan for all of their help.


# Symposium Schedule

| | |
|---|---|
| 8:00-9:00 a.m. | Breakfast, Registration (Physician's Promenade)<br>Student Poster Set-up (Alumni Hall) |
| 9:00-10:00 a.m. | Welcome: Drs. Chris Korey and Beth Meyer-Bernstein (College of Charleston) (Physicians Auditorium)<br>• Keynote: **Neurobiology in Drosophila-Small Brain, Large Potential**<br>Dr. Diane O'Dowd, Ph.D., Howard Hughes Medical Institute Professor<br>University of California, Irvine |
| 10:00-11:00 a.m. | Panel: Admissions to Graduate Programs (Physician's Auditorium)<br>• Karen Eippert, Director of Pre-professional Health Advising, C of C<br>• James Buggy, Ph.D., Dean of The Graduate School, USC<br>• John Kohler, M.D. Student, MUSC<br>• Jamie Van Etten, Ph.D. Student, University of Michigan<br>• Michael Guthrie, M.D./Ph.D. Student, MUSC |
| 11:00-11:15 a.m. | Coffee Break |
| 11:15-12:00 | Student Platform Presentations (Recipients of Student Travel Awards)<br>• **Tetrodotoxin Increases Retinal Ganglion Cell Neurite Branching *in vitro***<br>Kathryn Cole, Davidson College<br>• **Using Herpes Simplex Virus-1 to Over-express the Mu-Opioid Receptor in Afferent Neurons**<br>Rabiah Ali, University of South Carolina<br>• **The Effects of Acute verse Chronic Alcohol intake on Brain Glucose Utilization in Cannabinoid 1 Receptor Knockout Mice**<br>Lesley Dillard, Furman University: |
| 12:15-1:45p.m. | Lunch/Student Workshops (Rita Liddy Hollings Science Center)<br>• Session I: 12:15-1:00 p.m; Session II: 1:00-1:45 p.m. |
| 1:45-3:15pm | Poster Session at Alumni Hall (Randolph Hall) |
| 3:15-4:15 p.m. | ***The New Neuroethics*: Brain Function and Borderlands of Life, Death, and States in-Between**<br>Dr. Jerome Kurent, M.D., M.P.H., Professor of Medicine and Neurosciences, MUSC |
| 4:15 p.m. | Closing Remarks |

# Lunch Workshops

Each workshop discussion will last 45 minutes and will be presented twice (12:15-1:00pm and 1:00pm-1:45pm).

Workshop: **Scientific Writing**
Location: Science Center Room 106
Leader: Jennifer G. Schnellmann, Ph.D., E.L.S., Medical University of South Carolina

Workshop: **AMCAS Enhancement Tips**
Location: Science Center Room 218
Leader: Karen Eippert, Director of Pre-professional Health Advising, College of Charleston

Workshop: **Applying to Graduate School**
Location: Science Center Room 239
Leader: James Buggy, Ph.D. Dean of the Graduate School, University of South Carolina

Workshop: **Alternative Careers in Neuroscience**
Location: Science Center Room 227
Leaders: Christine Lauay, Ph.D. (Medical Writer), Yashmin Karten, Ph.D. (Technology Transfer)

Workshop: **Bioethics and the Personal Genome Project**
Location: Science Center Room 108
Leader: Dana Waring, Harvard Medical School

Workshop: **Neurons in Action 2 Interactive, Educational Software in Neurophysiology**
Location: Science Center Room 223
Leader: Ann Stuart, Ph.D. University of North Carolina-Chapel Hill

# SYNAPSE Participants

**Diane O'Dowd, Ph.D.:** Dr. Diane O'Dowd is an HHMI Professor in the Departments of Developmental and Cell Biology in the School of Biological Sciences and of Anatomy and Neurobiology in the School of Medicine at the University of California, Irvine. After receiving a B.S. in biology at Stanford University (1979), Dr. O'Dowd earned a Ph.D. at the University of California, San Diego (1985), where she was a graduate student in the lab of Dr. Nicholas Spitzer. She returned to Stanford as a postdoctoral fellow with Dr. Richard Aldrich where she first began studying ion channels in the fruit fly. Dr. O'Dowd was hired as an assistant professor at UC Irvine in 1989 and was promoted to full professor in 2001. For the past 20 years, her research lab at UCI has studied the activity of living neurons from the brains of both flies and mice. Using molecular genetic manipulations and whole cell electrophysiology, they are exploring the role of specific genes in regulating functional plasticity of developing and mature neural circuits.

Teaching is also an important facet of her career. Dr. O'Dowd has taught a number of large undergraduate classes at UCI and has participated in summer courses at both Cold Spring Harbor and Woods Hole. She was named a National Academies Education Fellow in the Life Sciences (2004-2005), was the recipient of UC Irvine's Distinguished Faculty Teaching Award (2005-2006) and became an HHMI Professor in 2006. With the resources provided by the HHMI Professorship, she and her colleagues are working to transform the relationship between teaching and research at UCI, a setting where research tends to dominate, and teaching is often viewed as a burdensome chore. They are developing strategies to help create dynamic learning environments in large biology classes that are compatible with the faculty building or maintaining successful research programs. Furthermore, her group is providing training in interactive teaching for graduate student TAs who teach discussions and training for postdoctoral fellows who mentor undergraduates in research.

**Jerome Kurent, M.D., M.P.H:** Dr. Kurent received his MD from the University of Cincinnati, and completed residencies in Medicine and Neurology at the Johns Hopkins Hospital. He completed fellowships in neuromuscular diseases and electromyography at the National Institutes of Health, and a geriatric medicine fellowship and MPH at Harvard. Dr. Kurent is Chair-elect of the American Academy of Neurology Ethics Section, and is immediate past-chair of the AAN Pain and Palliative Care Section. He is a Fellow of the American Academy of Neurology, and served on the National Hospice and Palliative Care Organization ALS Work Group. He is a Faculty Scholar of the Project on Death in America, and a Mayday Pain and Society Fellow. He is a member of the Medical University of South Carolina Ethics Committee and the South Carolina Medical Association Bioethics Committee. He is also a hospice physician. Dr. Kurent is Professor of Medicine and Neurosciences (Neurology) at the Medical University of South Carolina, where he participates in the Interdisciplinary ALS Clinic. He is a diplomate of the American Board of Hospice and Palliative Medicine; American Board of Pain Medicine; the American Board of Psychiatry and Neurology, and is a Certified Medical Director.

**Admissions Panel:**

**Karen Eippert**: Karen Eippert came to the College of Charleston in March, 2006. Prior to accepting her position as Director of Pre-professional Health Advising, she worked for 13 years at the Medical University of South Carolina in admissions, recruitment, and student development. Ms. Eippert earned a B.S. in Psychology from the College of Charleston and completed a master's degree in Career Development at John F. Kennedy University in the San Francisco Bay area. Her professional career has been devoted to helping students prepare for careers in health care.

**James Buggy, Ph.D.:** James Buggy has been with the Graduate School at the University of South Carolina since 2006, first as Associate Dean for Academic Affairs and currently as Interim Dean. He had previously served as Assistant Dean for Graduate Studies and Academic Director for the Biomedical Science and Nurse Anesthesia graduate programs for the USC School of Medicine and Department of Pharmacology, Physiology, and Neuroscience. He has been recognized with the USC School of Medicine Faculty Research Award in 1983 and School of Medicine Teaching Advancement Awards in 1997, 1998, 2000, 2003, and 2005. Previously, he was an instructor in Physiology and fellow at the Cardiovascular Center of the University of Iowa, earned the PhD in Psychobiology from the University of Pittsburgh, and was introduced to the science laboratory as a work-study undergraduate at the University of Pennsylvania.

**John Kohler:** John Kohler graduated *magna cum laud*e from the College of Charleston in 2007 with a B.A. in Spanish and a B.S. in Biology. He is currently a first year medical student at the Medical University of South Carolina.

**Jamie Van Etten:** Jamie Van Etten graduated *cum laude* from the College of Charleston in 2007 with a B.A. in Chemistry and a B.S. in Biochemistry. She also graduated from the Honors College. Jamie is currently a first year graduate student in the Chemical Biology program at the University of Michigan.

**Michael Guthrie:** Michael Guthrie graduated from the College of Charleston in 2006 with a B.S. in Biology. He also graduated from the Honors College. Michael is currently a first year student in the M.D./Ph.D. program at the Medical University of South Carolina.

**Workshops:**

**Jennifer G. Schnellmann, Ph.D., E.L.S.:** Jennifer G. Schnellmann is the Director of the MUSC Office of Scientific Editing and Publications at MUSC. She received a PhD in pharmacology and toxicology from the University of Arkansas for Medical Science in Little Rock, AR and completed a postdoctoral fellowship in neurotoxicology with the FDA at the National Center for Toxicological Research. She joined MUSC in 2001 as a board-certified editor in the life sciences where she serves as science/medical writer/editor.

**Christine Lauay, Ph.D.:** Dr. Christine Lauay received her Ph.D. in Biopsychology from the Department of Psychology of Cornell University in 2003. She did postdoctoral research at Northwestern University from 2003 to 2004, after which she began her career as a medical writer. Dr. Lauay is currently employed by Delta Pharma, Deerfield, Illinois. She works with pharmaceutical companies to write a variety of regulatory documents required during clinical drug development, including new drug applications, clinical study protocols, clinical study reports, and annual reports.

**Yashmin Karten, Ph.D.:** Yashmin Karten is Licensing Manager at the MUSC Foundation for Research Development. Her role is to develop and implement commercialization strategies for MUSC inventions and technologies. She was a postdoctoral researcher at the National Institute of Mental Health and at the University of Arizona and has several years of research experience in the field of neuroscience. She received her Ph.D. degree in Medical Biology from the University of Amsterdam in 2000.

**Dana Waring:** Dana Waring is a Research Assistant in the Social Sciences at Harvard Medical School. She got her BFA from Syracuse University and her MLA is Women's Studies from the Harvard University Extension School. Her current interests lie in the manner in which large-scale genome sequencing intersects matters concerning privacy, health & life insurance, science education and reproduction. At present, her work is focused on two main activities. Dana is creating materials and conducting educational events for high school and college students so that they can become familiar with the ethical, legal, and social issues (ELSI) regarding personal genetics, issues which will be important for them as individuals and as members of society. (www.pged.org)

**Schools Represented at SYNAPSE 2008:**

Belmont University (TN)
Clayton State University (GA)
College of Charleston (SC)
Davidson College (NC)
East Tennessee State University (TN)
Francis Marion University (SC)
Furman University (SC)
Georgia Institute of Technology (GA)
Harvard Medical School (MA)
James Madison University (VA)
Mary Baldwin College (VA)
Medical University of South Carolina (SC)
Midlands Technical College (SC)
University of North Carolina-Chapel Hill (NC)
University of South Carolina (SC)
Wake Forest University (NC)
Winthrop University (SC)
University of California-Irvine (CA)
University of Michigan (MI)

**Note:** For a complete list of presenters and attendees at SYNAPSE 2008 please go to the conference website: www.cofc.edu/synapse

# SYNAPSE 2008 Abstracts

**Student Platform Presentations:**

**Tetrodotoxin Increases Retinal Ganglion Cell Neurite Branching in vitro**

Cole KT, Ippolito DM, Cron CC, Lang K, Iordanou JC, MacDowell TL, Ruble JE, and Lom B
Biology Department & Neuroscience Program, Davidson College

Retinal ganglion cells (RGCs) are the only neurons that relay visual input from the eye to the brain. RGC axons form the highly organized optic nerve, which innervates the optic tectum of the midbrain in Xenopus laevis. Neuronal activity is involved in RGC axon refinement via NMDA-receptor-mediated mechanisms, implicating visual cues in the development of retinotopic connections. Moreover, RGCs are spontaneously active even before their axons synapse with tectal neurons. Thus we investigated the role of neuronal activity on initial RGC morphological differentiation by exposing RGCs to tetrodotoxin (TTX), which inhibits voltage-gated sodium channels, reducing neuronal activity. Xenopus retinal neurons were dissociated and cultured for three hours. Cultures were then randomly assigned to one of three experimental conditions: (1) Development in the absence of TTX (control), (2) low TTX (1 uM), and (3) high TTX (10 uM). Cultures were fixed six hours later and immunostained to identify RGCs. Fluorescence microscopy was used to image and quantify neurite length and branching. Neurons reared in the presence of TTX exhibited a significant dose-dependent increase in branching without affecting total neurite length or number of primary neurites. This increase in neurite branching in TTX-treated neurons suggests that neuronal activity plays an early role in RGC morphological differentiation. Similar enhancements in axon branching have been observed in TTX-treated Xenopus RGC axons in vivo. Thus, neuronal activity plays a role in determining the shape of Xenopus RGCs.

**Using Herpes Simplex Virus-1 to Over-express the Mu-Opioid Receptor in Afferent Neurons**

Ali R, Mohammed H, Raja SN, Wilson SP and Sweitzer SM
Department of Pharmacology, Physiology and Neuroscience, University of South Carolina

Neuropathic pain is a chronic condition that millions suffer with on a daily basis. Opioids are often administered at high dosages to help with the symptoms associated with chronic neuropathic pain. In this project, Herpes Simplex Virus-1 (HSV-1) was used to over-express the mu-opioid receptor (mOR) in primary afferent neurons. Four groups were included: a control virus encoding the E. Coli LacZ gene (SGZ), a HSV-1 encoding cDNA for mOR (SGMOR), a HSV-1 encoding cDNA for preproenkephalin (KPE), and a combination of the SGMOR and KPE viruses. HSV-1 viral constructs were administered to the left hindpaw via topical inoculation. Spinal cords were collected at 4 weeks post-infection to examine expression of the mOR using immunohistochemistry. Preliminary results show that when compared to the SGZ infected control group, the SGMOR infected mice have double the density of mOR immunoreactivity in the dorsal horn of the spinal cord. This increase in mOR immunoreactivity was observed in lamina I-III as compared to a more limited expression in lamina I-II in SGZ control infected mice. Also, infection with SGMOR+KPE virus increases mOR immunoreactivity in lamina I-III of the spinal cord. These results suggest that HSV-1 mediated viral vectors can increase expression of mOR in primary afferent neurons and may be used to enhance opioid analgesia in the treatment of chronic
neuropathic pain.

**The Effects of Acute verse Chronic Alcohol intake on Brain Glucose Utilization in Cannabinoid 1 Receptor Knockout Mice**

Dillard LA, Henderson P, Gottlieb K, Piyis YK, Michaelides M, Volkow ND, Thanos PK, and Rice O
Department of Psychology, Furman University

Alcohol has a number of physiological effects, none greater than its abuse potential. Studies have linked the behavioral effects of alcohol, including its abuse, with the brain's CB1 receptor - a part of the endocannabinoid system. The CB1 receptor is the most abundant neurotransmitter receptor in the brain and is localized in several areas, including the nucleus acumbens, amygdala, thalamus, superior colliculus, periaqueductal gray, and rostral ventromedial medulla. In this study, access to water for both the CB1 knockout (KO) and Swiss Webster (SW) mice was gradually decreased to one hour per day to simulate binge drinking. Animals were assigned to one of three doses: control (0 g/kg EtOH), low (0.5 g/kg EtOH), or high (1.5 g/kg EtOH). In the acute study, mice had access to ethanol only once (test day), while for the chronic study, they drank the same dose everyday for one month. On test day, mice were given 30 μCi of tritiated-2-deoxyglucose ([3H]2-DG) via the lateral tail vein immediately prior to them given access to ethanol for one hour. After the hour, blood glucose and EtOH concentrations were taken; the brains were extracted and later imaged using a Beta Imager. The acute study revealed that CB1 KO mice had significantly lower [3H]2-DG binding than their SW counterparts in both the striatum and hippocampus, two areas associated with the abuse of alcohol. Results from the chronic study are currently being analyzed and will be compared to the acute study. These results will be extremely valuable in the mapping of ethanol abuse pathways.

**Poster Presentations**

**1. Nicotine Facilitates Learning In Zebrafish, *Danio rerio***

Repasky R and McGrew L; Department of Biology, Belmont University

Losses in memory have been a devastating reality for millions of Americans, especially with modern increases in age and the onset of neurodegenerative diseases such as Alzheimer's disease. Studies have shown that in many model systems, nicotine increases memory. A recent addition to the group of standard models is the zebrafish Danio rerio, a small fish used traditionally in the studies of developmental biology and genetics. In our study, the fish were tested using a rapid-conditioning test to observe learning of side-preference in a tank. Previous studies used the salt nicotine ditartrate in their tests; however, in order to standardize results, this study used pure nicotine. Results revealed a dose-dependent curve, with optimum concentrations providing higher learning than control fish showed. We also determined that long-term exposure to nicotine produced results no different from fish not exposed to nicotine, suggesting the development of tolerance to the chronic presence of nicotine.

**2. The effect of perinatal DHT and E2 on copulatory behavior in the female musk shrew**

Siboni RB, Ewton TA, Jackson A, and Freeman, LM; Department of Biology, Mary Baldwin College

Testosterone (T) has three metabolic pathways: it can bind via androgen receptors, be aromatized to estradiol (E2, which acts via estrogen receptors) or it can be irreversibly reduced to dihydrotestoterone (DHT). Data suggest that altricial rodents require aromatization of T to E2 to differentiate copulatory behavior, while in primates rely more on the DHT pathway (Wallen and Baum, 2002). We studied the female musk shrew, an altricial insectivore in which female copulatory behavior is regulated by T. On postnatal days 1-5, female pups were injected with DHT, E2 or sesame oil vehicle. At 2-4 months, all animals were ovariectomized and implanted with small T capsules to ensure male-typical activational hormone levels. During behavior testing, the experimental shrew was placed in a test box with a stimulus female and observed for two 60-minute copulatory behavior tests by an experimenter blind to hormone treatment. After performing a 3X2 ANOVA (hormone treatment by trial), we found that there was a significant difference between DHT vs. E2 and DHT vs. controls in time to initiate sexual behavior and time until first mount. However, no significant differences were found with hormone treatment in total number of mounts, and overall mounting rate was less than half that previously reported in shrews injected with prenatal T (Freeman et al., 1998). Thus, the data suggest that while DHT may be an effective masculinizer in the initiation of male copulatory behavior, both metabolites may be necessary in the musk shrew to fully differentiate sexual behavior.

### 3. Sex Difference in Spatial Learning Found in Mice via Paddling Pool Maze

Cawthron B and Freeman L
Department of Psychology, Mary Baldwin College

The Paddling Pool Maze (PPM) has been proposed as an alternative to the Morris Water Maze (MWM) as a more suitable measure of spatial learning in mice (Deacon, 2004). The PPM is a circular, shallow water maze with up to 12 possible escape tubes on the perimeter (Fugger et al, 1998). Since males mice show better spatial learning than females in the MWM we hypothesized that they would show a similar sex difference in the PPM. During 8 training trials we gave the mice a 3-minute opportunity to find the only open escape route out of the 12 on the perimeter of the pool. We found no sex difference in escape time during training, $F(1,20) = 1.28$, $p = 0.27$. We moved the exit 120 degrees and gave the mice 3 probe trials. During the probe trials, females found the new exit significantly faster than the males, $F(1,20) = 17.179$, $p = 0.001$. Subsequent testing found that there was no sex difference in A) thigmotaxis in open field conditions, B) preference for a dry platform over water, and C) preference for entering a dark tube. Thus, we concluded that the sex difference could be attributed to spatial learning with males having stronger memory for either intra- or extra-maze cues, making it harder for them to learn the location of the new exit.

### 4. Long Term Exposure to Voluntary Exercise Decreases Cocaine's Reinforcing Efficacy

Schmidt KT, Iordanou JC, Mustroph ML, and Smith MA
Department of Psychology, Davidson College

Exercise has been shown to improve general physical and mental health and has been implicated as a potentially effective alternative to drug use. A handful of previous studies have shown that exercise impacts the self-administration of drugs. The present study examined whether chronic, voluntary exercise influenced the self-administration of cocaine using a progressive ratio schedule of reinforcement. Female, Long-Evans rats were obtained at weaning and separated into an exercise condition in which the home cages were modified with an exercise wheel attached or a sedentary control condition without an exercise wheel. Following 6 weeks in these conditions, rats underwent surgeries to implant intravenous jugular catheters and were tested in the cocaine self-administration procedure. After a period of acquiring the lever-pressing response on a fixed-ratio 1 (FR1) schedule, breakpoints on a progressive ratio schedule of reinforcement were determined for multiple doses of cocaine (0.0, 0.3, and 1.0 mg/kg/infusion) and compared between the two groups. Whereas the two groups did not differ in the number of days necessary to acquire the task on the FR1 schedule, the exercise group responded significantly less than the sedentary group for cocaine on the progressive ratio schedule at the 0.3 and 1.0 mg/kg/infusion doses. These results indicate that exercise decreases the reinforcing efficacy of cocaine and may be useful in therapeutic settings to decrease the likelihood of addiction.

### 5. Changes in Cutaneous Peptidergic and Nonpeptidergic Nociceptors Associated with Fetal Ethanol Exposure

Sanders D, McKelvy A, Guram G, and Sweitzer SM
Dept. of Pharmacology, Physiology, and Neuroscience, University of South Carolina, School of Medicine

As fetal alcohol syndrome disorder (FASD) occurs in 1-10 in 1000 births, there is a need for the complications of this disorder to be better understood. In particular, an animal model from our research group has shown that the peripheral neuropathy resulting from fetal alcohol exposure is characterized by decreased sensitivity to non-noxious mechanical stimuli and increased sensitivity to noxious thermal stimuli. It is thought that perhaps fetal alcohol exposure interferes, delays, or even inhibits proper peripheral nerve development and mylineation; however, the mechanism for this difference and its sensory consequences is not understood. In this study, we set out to examine the difference in the peripheral nerve endings in the skin of rats on a cellular level using immunohistochemistry. Specifically, we used the following antibodies: the PGP 9.5 which is a pan marker for cutaneous nerve endings, Isolectin B4 which stains for non-peptide containing, pain-sensing nerve endings, CGRP (Calcitonin Gene Related Peptide) which stains for peptide containing, pain-sensing nerve endings, and Neurofilament 200 which stains for A-Beta afferent, large diameter myelinated touch fibers. Preliminary results suggest a decrease in the PGP 9.5 immunoreactivity, and an increase in both CGRP immunoreactivity and IB4 binding in the animals exposed to alcohol versus the controls. This suggests a decrease in peripheral nerve endings in general, and an increase in both peptide and non-peptide containing pain-

sensing endings in the skin. These changes are likely to have a profound impact on how children with fetal alcohol exposure experience sensations like touch and pain.

### 6. Enhanced Capsaicin-Induced Thermal Hyperalgesia and Neuronal Activation in Fetal Alcohol Exposed Rats

Guram J, McKelvy A, and Sweitzer SM
Department of Pharmacology, Physiology and Neuroscience, University of South Carolina

In 9-10 out of every 1,000 human live births, the infant is born with fetal alcohol spectrum disorder (FASD). Among the many effects of FASD is increased sensitivity to painful thermal stimuli. We hypothesize that fetal alcohol exposure causes this alteration in sensitivity via changes in the activity of small diameter, unmyelinated primary afferent neurons that respond to temperature (C fibers). To test our hypothesis, we used a rodent model of FASD and examined responses to thermal stimuli and neuronal activation following application of the C fiber agonist capsaicin. Capsaicin is responsible for the "hot" taste of chili peppers. On postnatal day 21, an injection of 20μl of capsaicin was administered subcutaneously in the left plantar hind paw, activating C fibers. Ethanol-exposed animals exhibited increased capsaicin-induced thermal hyperalgesia as compared to control animals. Two hours post-injection, animals were perfused and spinal cords were isolated. Spinal cords were sectioned at 30μm and processed for c-fos using immunohistochemistry. C-fos is a marker of neuronal activity; higher levels of c-fos in the dorsal horn corresponded to greater neurotransmission from peripheral C fibers. We found an increase in the number of c-fos positive neurons in the superficial dorsal horn of ethanol-exposed rats as compared to control rats. These findings suggest that fetal ethanol exposure increases pain sensations that result from the activation of C fibers expressing the capsaicin receptor. Understanding the physiological basis of the FASD-induced increase in sensitivity to painful thermal stimuli is a fundamental first step in developing treatments for this disorder.

### 7. An Investigation of Cadmium Effects on Mitochondria Isolated from Mouse Brain

Polson AK, Dineley KE and Malaiyandi LM
Department of Biology, Francis Marion University

Cadmium ($Cd^{2+}$) is a relatively abundant environmental contaminant. Accumulation of $Cd^{2+}$ in nervous tissue causes neuropathy and while its cytotoxic effects are well-documented, it is unclear exactly how $Cd^{2+}$ kills cells. One potential mechanism involves inhibition of cellular energy production. In this study, we used fluorescence microscopy to monitor the effects of $Cd^{2+}$ on mitochondrial transmembrane potential in individual mitochondria isolated from mouse brain. Mitochondria were adhered to microscopy glass and loaded with rhodamine 123, a fluorescence indicator that collects in energized and respiring mitochondria with a robust transmembrane potential. We found that $Cd^{2+}$ at relatively low concentrations quickly and irreversibly dissipated transmembrane potential. $Cd^{2+}$ mitotoxicity was relatively potent and efficacious when compared to two other well-characterized mitotoxic metals, $Ca^{2+}$ and $Zn^{2+}$. These results demonstrate that $Cd^{2+}$ can substantially inhibit mitochondrial function, and provide important insight regarding the mechanism of $Cd^{2+}$-mediated neurotoxicity.

**8. A dose-response analysis of methylphenidate locomotor sensitization in adolescent D2-primed rats**

Hughes BA, Hughes AB, Sheppard AB, Cope ZA, and Brown RW
Dept. of Psychology East Tennesseee State University

Past studies from our laboratory have shown that neonatal quinpirole (dopamine D2/D3 agonist) treatment produces increases in dopamine D2 receptor sensitivity that persists throughout the animal's lifetime, a phenomenon known as D2 priming. A common drug typically used to medicate attention deficit-hyperacivity disorder (ADHD) is methylphenidate (Ritalin), a drug that has stimulant properties and abuse potential. In this study, we analyzed whether male and female adolescent rats neonatally treated with quinpirole will demonstrate locomotor sensitization to methylphenidate (MPH) when this drug is administered in adolescence. Rats were administered quinpirole from postnatal days (P)1-21. Beginning on P33, male and female rats were administered one of three doses of methlphenidate (MPH; 1, 3, or 5 mg/kg) or saline beginning on P33 every other day through P49. Results showed that females an approximate 100% increase in locomotor activity to the 5 mg/kg dose of MPH compared to males, and non D2-primed females demonstrated sensitization to the highest dose of MPH (5 mg/kg) and D2 priming blocked this sensitization. The lowest dose (1 mg/kg) produced locomotor suppression in females compared to controls. In males, both the 1 and 3 mg/kg of MPH produced locomotor suppression compared to controls, and males did not demonstrate sensitization to any of the three doses. Additionally, neonatal drug treatment did not affect the locomotor response to MPH in males. These results show that females are more sensitive the locomotor activating effects of MPH than males, whereas males are more sensitive the locomotor suppressing effects of MPH than females.

**9. Fluorescence Detection of MAO Activity in Mitochondria Isolated from Mouse Brain**

Tucker LN, Vernon PJ, Malaiyandi LM and Dineley KE
Department of Biology, Francis Marion University

Monoamine oxidase (MAO) enzymes degrade dopamine, serotonin, and norepinephrine and other monoamine neurotransmitters, and are important targets in the management of depression and psychiatric disorders. The two major isoforms, MAO-A and MAO-B, are both associated with the outer mitochondrial membrane, but their distribution varies depending on tissue. Specifically, MAO-A is abundant in liver, while both are found in brain. In this study, we developed a plate reader-based, fluorescence assay to detect MAO activity in mitochondria isolated from mouse brain. We used the indicator amplex red to monitor H2O2 production resulting from the oxidation of benzylamine and tyramine, and we tested MAO activity in the presence of various inhibitors. We have used these results to produce a relatively efficient and simple means of assaying MAO activity in a high-throughput fashion.

**10. A Comparison of Reactive Oxygen Species Production in Mitochondria Isolated from Mouse Brain and Liver**

Norris CA, Shupe JA, Malaiyandi LM and Dineley KE
Department of Biology, Francis Marion University

Mitochondria are a major source of reactive oxygen species (ROS), which includes free radicals such as superoxide and non-radicals such as hydrogen peroxide. At controlled levels, ROS participate in cell signaling and are probably beneficial. However, excess ROS are cytotoxic, and are thought to contribute to numerous clinical pathologies such as Parkinson's disease and amyotrophic lateral sclerosis (ALS). In the present study, we investigated ROS production in mitochondria isolated from mouse brain and liver using the fluorescence ROS sensor Amplex Red in a spectrofluorophotometer-based assay. Pharmacological manipulations revealed that ROS production in brain occurs by substantially different mechanisms compared to liver. Specifically, brain mitochondria produce ROS at high levels when supported by substrates metabolized by complex II of the electron transport chain, but ROS production was low when complex I substrates were used. In contrast, liver mitochondria produced ROS at relatively high levels regardless of substrate conditions. Additionally, brain ROS production was partly reliant on an intact transmembrane potential, whereas liver mitochondria produced ROS regardless of the transmembrane potential. Our results demonstrate that ROS production is differentially regulated in mouse brain and liver and that mitochondrial ROS production varies between different tissues.

**11. Histological and Behavioral Analysis of rAAV9-induced Rodent Model of Taupathy in Alzheimer's Disease**

Mustroph ML, Ramirez JJ, Klein RL and King M
Department of Psychology, Davidson College

One of two hallmark features of Alzheimer's Disease is tau neurofibrillary tangles. The project seeks to develop an accurate rodent model of AD taupathy, which can be used in the future to develop drugs that prevent tau pathologies in the brains of human Alzheimer's patients. The hippocampus is implicated in memory formation and one of the first brain areas affected in Alzheimer's Disease. Therefore, brain surgery targeting the hippocampus was performed on 20 male Sprague-Dawley rats. Ten rodents received 4 hippocampal injections (3 µl per injection) of replication-deficient adeno-associated viral vector serotype 9 (rAAV9) containing the gene for human tau with mutation P301L, which predisposes tau protein to hyperphosphorylation, increasing its tendency to aggregate into tangles. Ten control rodents received 4 hippocampal injections (3 µl per injection) of rAAV9 containing the gene for green fluorescent protein (GFP), a protein from the jellyfish Aequorea Victoria that naturally fluoresces under blue light and that reports expression. rAAV9s were obtained from the lab of Dr. Ronald Klein, Louisiana State University. Multiple subjects were tested daily on a Y-maze alternation task for six months. Preliminary findings indicate that spatial memory deficits occur in the group with the mutated tau gene. Throughout data collection, the researchers were blind to the conditions. Histology testing to confirm GFP and tau presence is ongoing in collaboration with Dr. Michael King, University of Florida.

**12. Progesterone Pretreatment Selectively Attenuates Reinstatement of Cocaine-Seeking in Estrous Female Rats**

Feltenstein MW, Byrd EA, Henderson AR, and See RE
Department of Psychology, College of Charleston; Department of Neurosciences, MUSC

Clinical research indicates a role of gender differences in mediating certain aspects of cocaine addiction, specifically, the rate at which dependence develops from casual use and the length of time spent abstaining from drug use. Preclinical studies have examined cocaine addiction with rat models of cocaine self-administration. These studies support the findings of clinical research and show that female rats respond at higher rates than males during self-administration, early extinction, and cocaine-primed reinstatement. These differences in behavior of cocaine use have been correlated with the female estrous cycle and plasma progesterone levels. Higher levels of progesterone during the proestrus phase correspond with decreased cocaine seeking behavior, whereas lower levels of progesterone during the estrous phase yielded higher cocaine-seeking behavior. Therefore, based on this inverse relationship between progesterone levels and cocaine seeking, the current study hypothesized that pretreatment with progesterone should yield diminished cocaine seeking behavior. Female Sprague-Dawley rats received jugular catheter surgery and were subsequently trained to self-administer cocaine (0.5 mg/kg per infusion). Cocaine seeking behavior was measured as a function of the rate of responding and the number of lever presses emitted by the rats in order to receive cocaine infusions during 2 hour daily sessions of self-administration. After the self-administration phase, the cocaine reinforcer was removed and rats no longer received infusions of the drug following lever pressing. This extinction phase continued for each rat until the rat extinguished the lever pressing behavior to a set criterion (i.e. 25 lever presses for 2 consecutive days). Following extinction, rats received an injection of cocaine (10 mg/kg, IP) before reinstatement testing began. To assess the effects of progesterone pretreatment on cocaine-primed reinstatement behavior, either progesterone (2 mg/kg, SC) or vehicle was administered to rats at 20 hours and 2 hours before reinstatement testing began. Responding on the active lever was measured throughout each of the three phases of the experiment. Vaginal smears and blood samples were collected during the extinction and reinstatement phases in order to determine estrous phase and measure plasma progesterone levels, respectively. This study found that females in the estrous phase of their cycle displayed increased responding on the previously cocaine-paired lever during early extinction and reinstatement. Furthermore, female rats in estrus, but not the other phases of the estrous cycle, who received progesterone pretreatment displayed attenuated lever responding during testing. The clinical applications of the results from this study may provide a better understanding of relapse in women in that it may depend on progesterone levels present during different phases of the menstrual cycle. Therefore, this research study could contribute to the development of a progesterone treatment strategy for women who are susceptible to cocaine relapse.

## 13. An Evaluation of the Health Belief Model as Applied to Helmet Use in College Undergraduates

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Helmet wearing practices and attitudes about helmet use were examined in 274 undergraduates who bicycle (Mean Age = 19.5 years, 78% females, 87% Caucasian). Participants completed a 57-item scale developed to assess components of the Health Belief Model (HBM; Rosenstock, 1974) and other items assessing demographics, bicycling habits and helmet use. We examined hypotheses that compared helmet wearers vs. non-wearers along dimensions of the HBM. According to the HBM, perceptions of vulnerability to injury and the severity of consequences associated with injury should relate positively to helmet use as do perceptions about the perceived benefits and exposure to cues to action. In contrast, perceived barriers are hypothesized to relate negatively to helmet use (Rosenstock, 1974). Consistent with previous research (e.g., Coron & McLaughlin, 1996), only 12.4% of the sample reported regular helmet use while bicycling. Overall, helmet users differed from non-users on HBM subscales [Omnibus F (10,197) = 20.63; p.< .001; Eta-squared = .512]. Consistent with model predictions, follow-up ANOVAs revealed group differences on all HBM subscales. These findings highlight the utility of the HBM in explaining helmet usage among college students and may help explain why traditional advertising campaigns or health promotion events are not more effective. Our findings highlight the need for interventions that inoculate individuals against negative peer pressure, encourage parents and significant others to insist on helmet wearing beyond childhood, and emphasize the emotional benefits of helmet use in addition to the risks associated with bicycling without helmets.

## 14. UNDERGRADUATE NEUROSCIENCE EDUCATION: USING IMPULSE AS A TEACHING TOOL

Jones LS , Allen L, Amin S, Baker D, Barrett S, Black LC, Blew M, Bonner HC, Bright LA, Desai R, Eubanks J, Goodlett B, Guram J, Harmon K, Juneja N, Jones N, Khaliq S, Khaliq Z, McClellan K, Meekins C, Montagu D, Nazir A, and Patel P
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The journal IMPULSE is in its fifth year of publishing articles both written and peer reviewed by undergraduates. Started in 2003 (Soc. Neur. Abs. 29:25.3; first issue 2004, Soc. Neur. Abs. 30:28.6), the journal provides an opportunity for students to publish their original research and review articles. It also offers undergraduate neuroscience faculty a mechanism to mentor their students through an authentic publishing experience, where the students write their own manuscript and experience the subsequent submission, peer review, and revision process. Further, faculty wanting to train students in the review process can work with IMPULSE review-team students at their institution and use the manuscript reviewing as a mechanism to teach experimental design, scientific writing, ethics in communications, etc. (see course suggestions in JUNE Spring 2006 Vol. 4, Issue 2 http://www.funjournal.org/results.asp?juneid=159). The IMPULSE review team has membership from around the world and serves the additional purpose of increasing undergraduate understanding of the international character of science. The journal is hosted at the University of South Carolina (http://impulse.schc.sc.edu), and is listed through the Directory of Open Access Journals. Growth in submissions has lead to creating a second Reviewer Training Site at Middlebury College, where Kim Cronise now serves as an additional Faculty Advisor. Supported by the SC Honors College.

**15. The effects of transcranial magnetic stimulation over the left motor cortex on pain perception in healthy adults**

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A number of neurostimulation methods of pain relief have been developed that act at various levels of the nervous system in order to relieve symptoms of chronic neuropathic pain. Transcranial magnetic stimulation (TMS) is a relatively painless and noninvasive form of stimulation that does not require sedation. There is evidence that fast (> 5 Hz) repetitive TMS (rTMS) over the motor cortex produces analgesic effects in both chronic pain patients and healthy adults. The exact frequency and intensity of stimulation to employ in order to achieve maximal pain relief, however, is not known. In an effort to elucidate this variable, this study examines the effects of various stimulus frequencies (1 Hz, 10 Hz, and 50 Hz triplets) and intensities (80%, 90% and 100% of resting motor threshold (rMT)) of rTMS over the motor cortex on pain perception in healthy adults. Pain measurements investigated include thermal pain thresholds, intensity and unpleasantness of supra-threshold pain stimuli, intensity of thermal wind-up pain, and mechanical pain thresholds. Results indicate that 10Hz TMS at 80% rMT seem to inhibit hyperalgesic effects significantly during thermal pain testing. However, perhaps due to a limited data set, it appears that motor TMS with all other intensities and frequencies did not produce any reliable effects on pain perception in healthy adults.

**16. Nicotine-conditioned hyperactivity in adolescent male and female D2-primed rats.**

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The aim of this study was to determine the ability of a nicotine-conditioned context to elicit locomotor hyperactivity in a neonatal quinpirole animal model of psychosis, and whether this conditioned hyperactivity could be blocked by the D2 antagonist eticlopride. Sprague-Dawley rats were treated with either saline or the dopamine D2 receptor agonist quinpirole from postnatal days (P) 1-21 to create priming of the dopamine D2receptor, a phenomenon that we have shown persists throughout the animal's lifetime. Beginning on P33, animals were injected i.p. with either nicotine (0.5 mg/kg), the D2 antagonist eticlopride followed by nicotine, or saline and placed into the arena 10 min after injection every other day through P49. A non-paired group was included. Results showed that, D2 priming blocked the typical initial hypoactivity produced by nicotine, and these animals also sensitized to nicotine more rapidly than controls. Eticlopride blocked sensitization to nicotine in both D2-primed and non D2-primed rats. On P50, a portion of these animals were administered a drug-free test in which rats were given saline before being placed into the locomotor arena. Interestingly, control animals administered nicotine demonstrated conditioned hyperactivity on the drug free test that was blocked by eticlopride, but eticlopride did not affect nicotine-conditioned hyperactivity in D2-primed rats. This result indicates priming of the D2 receptor was able to overcome D2 receptor blockade in adolescent rats. Further studies are analyzing the role of nicotinic receptors in this phenomenon, as collaborators have shown that D2 priming produces alpha7 nicotinic receptor upregulation.

**17. Nicotine sensitization in adolescent Beta Arrestin-2 knockout mice: Correlations with BDNF**

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Beta Arrestin-2 is a protein that regulates hydrolysis of the G-protein and is co-localized with the dopamine D2 receptor. In this study, 3-4 week old adolescent BA-2 KO and wild type C57/B6 mice were administered either nicotine tartarate (s.c, 0.5 mg/kg) or saline 10 min before being placed into the locomotor arena on each of seven (Experiment 1) or 14 (Experiment 2) consecutive days. A drug-free abstention period of seven days followed nicotine sensitization in each experiment, at the end of which animals received a nicotine challenge (0.5 mg/kg free base). Experiment 1 results showed that BA-2 KO mice were slightly hypoactive and did not demonstrate sensitization by day 7, whereas wild type controls did not demonstrate an increase in activity as compared to saline-treated wild types by day 7, but did show an increase in activity over the 7 days of nicotine treatment. On the nicotine challenge, BA2 KO blocked expression of nicotine sensitization. In Experiment 2, BA-2 KO mice demonstrated sensitization although not the levels of the controls. On the challenge, beta arrestin-2 again blocked expression of sensitization. Brain tissue was taken in both experiments for analysis of Brain-derived neurotrophic factor (BDNF) in the nucleus accumbens, and

showed that nicotine produced a significant increase in BDNF which was blocked by the knockout of beta arrestin-2. These results show that beta arrestin-2 plays a more important role in expression as compared to induction of nicotine sensitization, and blocks nicotine-induced increases in BDNF in the nucleus accumbens.

### 18. Amphetamine sensitization in a rodent model of psychosis

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This study was designed to analyze the effects of amphetamine on locomotor sensitization in a rodent model of psychosis developed in our laboratory. Past studies have shown neonatal quinpirole (dopamine D2/D3 agonist) produces a significant increase in dopamine D2 receptor sensitivity that persists into adulthood, a phenomenon known as D2 receptor priming. An increase in D2 receptor sensitivity is consistent with several behavioral disorders, including schizophrenia. Rats were administered quinpirole (1 mg/kg) or saline from postnatal days 1-11 and raised to adulthood (postnatal day 60). Beginning in adulthood, rats were administered d-amphetamine sulfate (1 mg/kg) or saline every other day for 14 days, resulting in a total of seven exposures to the drug. Approximately 10 min after drug injection, rats were placed in a locomotor arena and overall activity was analyzed. Results showed that D2-primed rats receiving amphetamine demonstrated a significant increase in locomotor activity across all seven days of testing relative to all other groups. Controls receiving amphetamine also demonstrated a significant increase in activity over days, demonstrating sensitization. Interestingly, D2-primed rats given saline demonstrated a lack of ability to habituate to the environment, and actually increased activity over days. Seven to fourteen days after locomotor sensitization testing was complete, cerebrospinal fluid samples were taken via microdialysis from the nucleus accumbens core to be analyzed for dopamine levels, and results showed that D2-primed rats demonstrated a significant 5-fold increase in dopamine levels compared to controls administered d-amphetamine. Supported by NIH grant 1 R15 DA 020481-01 to RWB.

### 19. Differential Effects of α-MSH on Social Behavior in Mice Depending upon the MC1R Receptor

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Research supports that α-MSH (melanocyte-stimulating hormone) modulates pain sex-dependently. This sex-dependent mechanism involves the abundance of melanocortin-1 receptors (MC1R) in the periaqueductal gray (PAG). Because this region influences social behavior, the relationship between α-MSH and the MC1R, was studied with social interactions in mice; female estrous cycle state was also considered. Possible α-MSH effects in the absence of the MC1R propelled testing on MC1R mice. 160 adult, naïve Swiss Webster and MC1R knockout mice were randomly assigned a partner of the same strain and opposite sex to one of eight experimental conditions, which varied based on drug and estrous state. Based on vaginal appearance, females were verified as estrus or non-estrus. Following intracerbroventricular (ICV) injections of α-MSH or saline, the pair was placed in a social interaction box. The following behaviors were quantified: locomotor activity (line crosses), flees, tailfollows, anogenital investigation, frontal investigations, rears, self-groomings, mountings, initiate contacts, and squeaks. MC1R females displayed greater sensitivity to α-MSH than SW females. α-MSH showed little or no effect in SW or MC1R males. α-MSH affected social, rather than non-social, interactions.

**20. Treatment of injured peripheral nerves with chondroitinase ABC leads to improved functional recovery**

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Degrading chondroitin sulfate proteoglycans (CSPGs) with the bacterial enzyme, chondroitinase ABC (ChABC), enhances axonal regeneration after nerve transaction and repair. However, whether enhanced regeneration leads to improved functional recovery is not known. In this study, we investigate the extent of functional recovery in ChABC-treated rats. The sciatic nerve was transected unilaterally and the distal stump treated with ChABC prior to conventional end-to-end repair with fibrin glue. Beginning two weeks later, video records (120fps) of level rat treadmill locomotion at a speed of 11m/m were obtained once a week for the next ten weeks. Kinematic analysis of hindlimb movements during treadmill locomotion was used to evaluate functional recovery in ChABC-treated animals and compared to untreated controls. Following sciatic nerve transaction, muscular control of ankle joint movement is lost. No ankle flexion occurs during the swing phase (F epoch), there is a larger and more prolonged period of ankle flexion during the early stance phase (E2 epoch), and there is very little ankle extension to achieve limb push off during late stance (E3 epoch). Rats attempt to compensate for this lack of control using greater hip flexion during swing, less knee flexion during E2 and hip flexion instead of extension during E3. In ChABC-treated rats, ankle flexion during F epoch is restored, beginning as early as four weeks after nerve repair surgery, and compensatory changes at the hip and knee are reduced or eliminated. In untreated controls, none of these signs of functional recovery was noted, even ten weeks post-operatively. Chondroitinase ABC treatment of injured peripheral nerves leads to enhanced axon regeneration and also improved functional recovery.

**21. The Role of AMPA/Kainate and Dopamine Receptors on Reinstatement of Heroin Seeking**

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The nucleus accumbens is a crucial structure in the neural circuitry of drug reward and addiction. It is known that both dopaminergic and glutamatergic innervations of this area play important and necessary roles in the modulation of several drug seeking behaviors. In this study we examined the effect of antagonism upon dopamine and AMPA/kainate receptors in the nucleus accumbens core (NAc) and how the blockade of these receptors relates to reinstatement of heroin seeking behavior in rats. We introduced the AMPA antagonist CNQX and the dopaminergic antagonists fluphenazine, sulpiride, or SCH-23390 and assessed the behavioral effects of this receptor blockade upon heroin primed reinstatement. We found that each of the antagonists significantly blocked heroin primed reinstatement compared to controls, thus indicating that both AMPA/kainate and dopamine receptors in the NAc are necessary for reinstatement of heroin seeking in rats. This shows that both glutamatergic and dopaminergic pathways within the nucleus accumbens play key roles affecting heroin reinstatement.

**22. Neuroprotective efficacy of estrogen and calpeptin is enhanced in motoneurons co-cultured with astrocytes exposed to glutamate-toxicity**

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Estrogen mediated neuroprotection is well established in single cell cultures, but, no data has yet been presented examining the effects when motoneurons are co-cultured with astrocytes. Astrocytes are an integral part of the nervous system that serves as supporting cells to the motoneurons. In the case of injury or neurotrauma, they form glial scars, which can hinder cell growth and prevent axon penetration. This study focused on the neuroprotective role that astrocytes offer to the motoneurons in glutamate induced neurotoxicity. Co-culturing astrocytes with motoneurons enabled the motoneurons to withstand twice the concentration of glutamate compared to motoneurons cultured alone, as shown by quantitative cell viability assay using MTT and morphologically by In Situ Wright staining. Estrogen and calpeptin were also shown to prevent glutamate-induced cell death in co-cultures. Calpeptin rendered protection via inhibition of the neutral protease calpain, which was evident from attenuation of active m-calpain and downstream active caspase-3 expression in the co-cultures using Western blot. The mechanisms by which estrogen protected the motoneurons in co-cultures remain to be elucidated by further studies.

**23. Characterization of Drosophila Palmitoyl protein-thioesterase 1's role in Cellular Trafficking**

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Infantile Neuronal Ceroid Lipofuscinosis (INCL) is a pediatric neurodegenerative disease caused by mutations in the human CLN1 gene. CLN1 encodes palmitoyl-protein thioesterase 1 (PPT1) suggesting an important role for the regulation of palmitoylation in normal neuronal function. To further elucidate Ppt1 function, we recently performed a gain-of-function modifier screen in Drosophila using a collection of enhancer promoter transgenic lines to suppress or enhance the degeneration produced by over-expression of Ppt1 in the adult visual system. Modifier genes identified in our screen connect Ppt1 function to synaptic vesicle cycling, endo-lysosomal trafficking, synaptic development, and activity-dependent remodeling of the synapse. Our results compliment recent work on mouse Ppt1-/- cells that shows a reduction in synaptic vesicle pools in primary neuronal cultures and defects in endosomal trafficking in human fibroblasts. We have now followed up on our results by examining general endocytic mechanisms and synaptic function in Drosophila Ppt1 mutants. Like human fibroblasts, we have shown that larval garland cells have defects in general endocytosis. Furthermore, we have characterized gain- and loss-of-function genetic interactions between Ppt1 and the Drosophila dynamin gene, shibire. Finally, we will present developmental and functional analysis of Ppt1 mutant neuromuscular junctions. Taken together our work suggests that trafficking defects may be one of the underlying cellular causes of neuronal dysfunction in INCL patients.

**24. Role of β-endorphin in behavioral despair, stress, and anxiety**

**Smith, CT, Cloonan, G, Lee, A, and Grisel JE**
Department of Psychology, Furman University

β-endorphin , a natural opioid, is released in response to psychological stress as well as physiological stressors, such as alcohol (EtOH) and is believed to play a role in the regulation of the effects of stress and EtOH. To investigate the role of β-endorphin in stress and its relation to alcohol consumption, a line of male and female transgenic β-endorphin mice (Rubinstein et al., 1996) having different basal levels of the opioid in their brains were subjected to two tests—modified versions of Porsolt's (1977) Forced Swim Test (FST) and Steru's (1985) Tail Suspension Test (TST)–of behavioral despair (akin to depression in humans) after being given an intraperitoneal injection of EtOH or saline. These tests measure behavioral despair and anxiety through the amount of mobility or immobility mice show when placed into aversive environmental situations. Adrenal glands were also removed from naïve male and female KO, HT, and C57/WT β-endorphin mice and weighed to determine any fundamental differences in adrenal gland size (and, thus, physiological responses stress) among the three genotypes. Behavioral test results have been inconclusive so far. FST and TST tests should produce similar results since they supposedly measure the same concept (behavioral despair). This has not been the case in our initial set of experiments. Our preliminary behavioral findings do, however, suggest an interaction among EtOH, β-endorphin levels, and stress. This relationship seems to vary between different sexes and strains. The results from the adrenalectomies suggest that adrenal gland weights differ significantly among the three strains of mice with the KO mice having the largest adrenal glands, probably due to the fact that they do not produce β-endorphin , resulting in a chronically stressed state. Additional research will need to be conducted to allow us to gain additional insight into the relationship between β-endorphin , EtOH, and stress.

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## 25. Investigating the Role of β-endorphin in Mediating Alcohol Reward Using *in vivo* Microdialysis

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**Rationale:** Alcohol reward has been implicated in dopamine and, to a lesser degree, glutamate release in the nucleus accumbens.
**Objectives:** This study hoped to elucidate the relationship between the endogenous opioid β-endorphin, which has been implicated in stress reduction and feelings of euphoria, and the release of dopamine and glutamate in the nucleus accumbens of transgenic mice possessing different "levels" of β-endorphin production capacity in response to alcohol administration.
**Methods:** In vivo microdialysis and HPLC was used to assess the relative concentrations of extracellular glutamate and dopamine in the nucleus accumbens of β-endorphin knock-out (KO), heterozygous (HT), and wild-type control (C57) mice at normal, baseline conditions and after acute ethanol administration.
**Results:** Results are currently preliminary and tentative as the glutamate HPLC assay has currently been performed on only one of each strain of mouse. Glutamate levels appear to be fairly stable in the C57 mouse and most variable in the HT mouse. The KO mouse showed a marked decrease in glutamate levels after ethanol administration while the HT mouse's glutamate levels spiked, then declined after receiving the drug.
**Conclusions:** Clear conclusions cannot be drawn based on our current results. The addition of more glutamate data, which is forthcoming, and the beginning of dopamine HPLC analysis on all microdialysis samples collected will assist in elucidating a possible relationship between β-endorphin, glutamate, dopamine, and the induction of "reward" or "pleasure" in the nucleus accumbens as a result of alcohol administration. Determining the relationship between these varied neurological components will assist in creating a possible explanation for alcohol addiction and may offer insight into future areas of research related to the treatment of alcoholism.

## 26. Differences in Analgesic Effects of Lidocaine HCl and Lidocaine Docusate Topical Solutions in Mice

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Topical pain medications are a commonality today - therefore, finding one that is more effective at blocking pain is important. This study tests two formulations of Lidocaine - a hydrochloride solution and a docusate solution - both diluted to proper concentration in DMSO. Lidocaine HCl is a commonly used form of lidocaine and acts as the control test compound. We predict that the docusate solution will have a different effect from the hydrochloride solution. We tested three concentrations of each solution. Subjects were male and female Swiss-Webster mice (n=45). Subjects were tested for analgesic effects by the tail flick method. Talk flicks were measured 10 and 20 minutes prior to a minor thermal burn injury to get a baseline reading. They were measured again twice post-injury, then treated with one type and dose of medication. Then tail flicks were measured for four hours at even intervals. Data was analyzed for between groups differences, area under the curve, and dose-response relationships. The docusate solution had a significantly greater effect than the hydrochloride solution. It acted faster and blocked pain longer. Tail flick latencies were as much as two fold higher in mice treated with the docusate solution. This shows good promise in potentially increasing the analgesic effects of topical pain ointments by using a different formulation.

## 27. Effect of Social Environment on Estrogen Receptor Alpha in Prairie Voles

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Social environment, particularly isolation, has a significant impact on neuroendocrine responses and social behaviors. Prairie voles are socially monogamous rodents (displaying life long pair bonds and biparental care) and may be particularly sensitive to the effects of isolation relative to other rodent species. Isolated prairie voles (Microtus ochrogaster), demonstrate deficits in individual recognition and discrimination and are more reactive to social stressors. Isolation also affects central vasopressinergic responses in this species, particularly in females. Species comparisons among rodents (including voles) demonstrate a relationship between estrogen receptor alpha (ERα) and the degree of prosocial behavior. This association is particularly notable within discrete limbic system nuclei. In the present experiment, juvenile prairie voles were housed with a sibling, stranger or in isolation for three weeks following weaning (at 21 days of age). ERα expression was then quantified in the central nervous system using immunofluorescence. The number of neurons containing ERα were counted in the medial preoptic area (MPOA), medial amygdala (MeA), bed nucleus of the stria terminalis (BST) and ventromedial hypothalamus (VMH). Isolate females displayed significantly fewer ERα labeled cells in MPOA and MeA areas compared to the stranger housed females. ERα expression in the MPOA and MeA of males was not significantly different among housing conditions. This suggests that females may be more reactive to the stress of isolation in this species

## 28. Gender Differences In Stimulant-Mediated Smoking Behavior

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Previous studies suggest that a large majority of stimulant abusing and dependent individuals smoke cigarettes. Conversely, cigarette smokers are more likely to use illicit drugs like stimulants. However, cigarette and stimulant co-use remains poorly researched. While the results of previous studies indicate that sex may differentially influence physiological and behavioral responses to cigarette smoking, the literature is mixed regarding whether males and females are differentially sensitive to the acute effects of stimulants. It is thus unclear whether males and females are differentially sensitive to stimulants, and what effect this could have on smoking behavior. In this experiment, the influence of sex on the acute effects of several doses of methylphenidate (0, 10, 20, and 40 mg) and ad libitum smoking was examined using a retrospective analysis of data from two previously published studies using identical procedures and measures. One hour after ingesting drug, participants were allowed to smoke and consume food and non-caffeinated beverages ad libitum for four hours. Primary dependent measures were number of cigarettes smoked, number of puffs taken, expired CO level, and number of calories consumed. Significant dose by gender interactions were observed for peak carbon monoxide levels (men > women). Significant main effects of gender were observed for subject ratings of Willing to Pay For Drug (women > men). As expected, methylphenidate dose-dependently decreased measures of food consumption, and dose-dependently increased heart rate, blood pressure, and several measures of cigarette smoking rate. This analysis indicates that men and women differed only in the biological measure of cigarette intake as a function of methylphenidate dose but not on other measures of cigarette smoking behavior.

## 29. Fluorescence Methods for Detecting Heavy Metals in Solution

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Fluorescent probes have broadened our understanding of the role of metals such as Ca2+ and Zn2+ in cell biology. However, one critical weakness of ion-sensitive fluorophores is their tendency to respond to a variety of species. Thus metal contamination often leads to spurious results. In particular, Zn2+ and Cd2+ are similar in chemical properties, and consequently are difficult to distinguish biological samples. In the present study we tested a variety of fluorescent probes in order to establish a spectrofluorophotometer-based assay that can selectively detect $Zn^{2+}$ and $Cd^{2+}$ in KCl-based buffered solutions. We examined the spectral responses that $Zn^{2+}$ and $Cd^{2+}$ elicit from the ratiometric probes fura-2, fura-2FF, mag-fura-2, and the intensiometric probes Calcium Green-5N, FluoZin-3, and Newport Green. We found that the mag-fura-2 responses to $Zn^{2+}$ and $Cd^{2+}$ differed substantially in both quantitative and qualitative aspects.

Specifically, $Zn^{2+}$ resulted in an isosbestic point around 346nm, while the isosbestic point for $Cd^{2+}$ was 353nm. Calcium Green-5N responded strongly to $Cd^{2+}$ but only weakly to Zn2+, with no spectral shift in either case. The spectral compatibility between mag-fura-2 and Calcium Green-5N allowed us to combine both dyes for simultaneous measurements, which increased resolution. We then added physiological amounts of $Ca^{2+}$ and $Mg^{2+}$ to the KCl solution in order to test the dual-dye method in a more biologically relevant situation. In this final medium, nanomolar $Cd^{2+}$ produced a mag-fura-2 isosbestic point of 356nm and a strong response to Calcium Green-5N, while nanomolar $Zn^{2+}$ resulted in a mag-fura-2 isosbestic point of 345nm and a weak Calcium Green-5N response. In summary, the dual-dye approach allows sensitive and discerning detection of $Zn^{2+}$ and $Cd^{2+}$ in biologically relevant solutions. Future experiments will determine the viability of this method for detecting metals in whole, living cells.

### 30. Computational Model of CA1 Pyramidal Neurons in the Hippocampus of Mice

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In order to study the mechanism underlying the electrophysiological behavior of CA1 pyramidal neurons in the hippocampus of wild-type mice, a conductance-based single-compartment computational model is utilized. Fundamental Hodgkin-Huxley based currents are incorporated into the model as well as a glutamate ionotrophic receptor, NMDA (N-methyl-D-aspartic acid) because they are important for the normal electrophysiological behavior of these neurons. The model reproduces essential features of the hippocampal biological neuron and offers the basis for a large-scale study of networks involving CA1 hippocampal pyramidal cells.

### 31. Catalytic Interlesion Interval Facilitates the Emergence of Long-Term Potentiation

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In rat brains, damage to one entorhinal cortex (EC) destroys the ipsilateral perforant pathway and thereby denervates the ipsilateral dentate gyrus. However, the crossed temporodentate pathway (CTD), which links the intact EC of the contralateral hemisphere to the denervated dentate, sprouts and has been shown to restore lost memory function. Two stage lesions of the entorhinal cortex accelerate CTD sprouting. In other hippocampal projections, interlesion intervals of 4 to 13 days have been found to induce the greatest amount of sprouting. We explored the ability of a 12 day interlesion interval to catalyze and accelerate the CTD's ability to support long term potentiation. Electrophysiological data from rats with 6 or 12 day interlesion intervals were examined to determine whether the sprouted CTD could support long term potentiation. Our results indicated that the 12 day interval produced a sprouted pathway that was able to support criterion-based long term potentiation while the 6 day interval did not. This finding suggests that a 12 day interlesion interval more effectively prepares the CTD to support long term potentiation.

### 32. The effects of (+)- and (-)-cyclazocine on cocaine-induced locomotor activity

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Cocaine is a highly addictive substance whose behavioral effects are linked with increased dopamine concentrations in the mesolimbic dopaminergic pathway. Activity at opioid receptors and sigma receptors can modulate cocaine's effects. Benzomorphans are a class of morphine derivatives whose (+) and (-) isomers interact with opioid and sigma receptors, respectively, and therefore may influence cocaine's effects. The current studytests the combined effects of cocaine and two benzomorphans, (+)- and (-)-cyclazocine, on locomotor activity in male Long Evans rats. Consistent with our hypotheses, (+)-cyclazocine (a sigma receptor agonist) increased the effects of cocaine in an additive manner, whereas (-)-cyclazocine (an opioid receptor agonist) increased the effects of cocaine in a synergistic manner. These data indicate that benzomorphans can serve as useful tools to explore cocaine's interaction with the opioid and sigma systems.

**33. Chronic Excercise Enhances Cocaine Conditioned Place Preference**

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Center for Interdisciplinary Studies, Davidson College

The purpose of this study was to determine whether chronic exercise alters sensitivity to the conditioned rewarding effects of cocaine. Female rats were obtained at weaning and randomly assigned to either sedentary or exercise conditions. After 6 weeks under these conditions, the effects of cocaine were examined in the conditioned place preference procedure. Cocaine produced a dose-dependent conditioned place preference in both groups of rats. Exercising rats were more sensitive than sedentary rats to cocaine in this procedure, and this effect was most pronounced at the highest dose of cocaine. These data suggest that chronic exercise increases sensitivity to the conditioned rewarding effects of cocaine.

**34. The Effects of Cue Induced Reinstatement of Heroin Seeking on Glutamate Levels in the Nucleus Accumbens Core**

Young, LM
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Relapse is a prevalent and severe problem among opiate addicts in treatment. Exposure to drug associated environmental cues often trigger reinstatement of drug seeking. The transmission of glutamate to the Nucleus Accumbens core aids in the reinstatement of cocaine seeking. These findings suggest an association between cue induced reinstatement of heroin seeking and glutamate levels in the Nucleus Accumbens core. Heroin self administration sessions involving light/tone cues paired with heroin infusions for two weeks were performed with one group of subjects, along with a yoked/control group receiving noncontingent saline, followed by two weeks of extinction training. Upon completion of self administration, microdialysis was performed in the Nuclues Accumbens core during cue induced reinstatement of heroin seeking for both the self administration and yoked groups. An increase in glutamate was found during reinstatement of the self adminstration group but not in the yoke group.

**35. Investigation of the Effects of LPA on Chicken Retinal Growth Cone Collapse**

Le MA, Billings DL, and Birgbauer EC
Department of Biology, Winthrop University

Visual system development is dependent on axon guidance in retinal ganglion cells (RGCs). In chicken embryonic retina, axons of RGCs extend and travel through various tissue to reach their target in the brain, the tectum. These axons are guided by growth cones which respond to extracellular cues, mediating their directional pathway. Lysophosphatidic acid (LPA), a lipid signaling molecule, is known to play a role in a variety of biological pathways, including neurons. We are investigating LPA as an inhibitory guidance cue on RGC growth cones in the retinal system. Six day-old embryonic chicken retinas were cut into explants and neurite outgrowth was achieved using laminin as a substrate. LPA was then administered to growth cones at various concentrations ranging from 1 nM to 1 μM. Observance of growth cone morphology reveals that LPA induces an in vitro inhibitory response of retraction or collapse. Preliminary results suggest that this occurs in a dose-dependent manner. LPA is known to signal through five G-protein coupled receptors (GPCRs) to induce an intracellular G-protein signaling cascade. Thus, we will continue our investigation with the identification of the G-protein coupled cellular pathways activated by LPA receptors. In addition, we are studying the expression of the LPA receptors at different stages of chicken retinal development to examine their role in RGC axon guidance.

**36. Wake up and smell the caffeine: Zebrafish locomotor activity following caffeine exposure**

Coulter S[1], Pellicoro L[3] and Hurd MW[1,2]
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Originally used in many cultures for ceremonial purposes or as a daily stimulant, caffeine has become one of the most ubiquitous drugs used in the western world. In particular, caffeine has become a regularly overused stimulant in the United States. Zebrafish have served as a model organism for the study of development for more than 30 years. These animals have also been useful for the study of effects of cocaine, nicotine, ethanol and melatonin on behavior and development. In this study, we examined the acute effects of caffeine on zebrafish locomotor activity. Animals were exposed to either 0.01 or 1.0 mg/L of caffeine or a control condition for 5 minutes. Fish were subsequently transferred to an observational arena and locomotor activity was assessed by manually counting the number of line crossings on a grid below the arena. Additionally, we used the Noldus® Ethovision system to digitally image the activity of fish in the arena. We hypothesized that caffeine exposure would stimulate activity in these fish. However, caffeine exposure produced a dose dependent decrease in locomotor activity. At the 0.01mg/L dose (N=15), we saw a decrease in mean activity counts of 18% compared to the control condition. A dependent t-test indicated that this reduction in activity was significant ($t$ (15) = 3.18, $p < 0.003$). In a second group of animals exposed to 1.0 mg/L (N=16), we saw a 34% decrease in mean activity counts. A dependent t-test indicated that this reduction in activity counts was significant ($t$ (15) = 2.38, $p < 0.015$). An analysis of these data using the Noldus system is currently in progress. Future experiments will examine conditioned place preference following caffeine exposure.

**37. The absence of thrombomodulin expression in developing avian spinal cords on E10**

Ammay KL, Meuleners C, and Turgeon V
Neuroscience Department, Furman University

Thrombin is a serine protease involved in coagulation via activation of the PAR-1 receptor. It has also been detected within the developing murine and avian CNS and is believed to play a role during development. Here it has primarily been found to have destructive effects, including increased apoptosis in avian motoneurons in the lumbar spinal region. Thrombomodulin, a transmembrane protein known to regulate the coagulant properties of thrombin, has also been detected in the CNS of murine embryos. Therefore, this study investigated the presence of thrombomodulin in the developing avian spinal cord. Experimental embryos were given 200 ƒ_L of 100 ƒ_M SFLLRNP (an artificial PAR-1 activator) daily from E5 to E9 while control embryos were given equal amounts of 1X PBS; on E10 the embryos were sacrificed. The spinal cords were prepared for thrombomodulin immunohistochemistry. Thrombomodulin was not detected. This finding suggests a different mechanism for regulating thrombin in the avian developmental system, such protease nexin-1, which is a potent protease inhibitor. Perhaps thrombin regulation is less complex in avian developmental systems. It is possible that mammals require multiple regulatory compounds to monitor thrombin activity, whereas birds utilize a more simple mechanism involving protease nexin-1.

**38. Changes in the Hippocampal Expression and Distribution of the KA2 Subunit of the Kainate Receptor in Epileptic Rats**

Barrett SC, Stanley EM, and Mott DD
Department of Pharmacology, Physiology and Neuroscience, University of South Carolina Columbia

Temporal lobe epilepsy (TLE) is a brain disorder characterized by recurring spontaneous seizures which can result from a brain injury. The seizures usually originate in the hippocampi. Glutamate is an excitatory neurotransmitter. Glutamate receptors can be divided into several subtypes, including kainate receptors (KARs). KARs are composed of a variety of subunits which can include KA2. KARs play a role in the initiation and spread of seizures, and the presence of KARs containing KA2 can increase the risk of seizures. However, seizures can increase the expression of KA2. We hypothesize that the increased presence of KA2 during epilepsy predisposes the brain to seizures. We examined changes in the hippocampal expression of KA2 in epileptic Sprague-Dawley rats. Rats were made epileptic using the pilocarpine model of TLE. This model induces status epilepticus (a prolonged seizure) via intraperitoneal injection of the convulsant pilocarpine. The status causes brain trauma, which leads to the development of epilepsy. We performed immunohistochemistry to assess changes in the expression and distribution of KA2 in the hippocampi of

rats which had been epileptic for 12 weeks. A better understanding of the changes in KA2 expression and distribution during epilepsy could lead to a novel therapy for TLE.

**39. A role for neural activity in Xenopus laevis retinal ganglion cell dendrite morphogenesis in vivo.**

Garren E, Ruble J, Watson F, and Lom B
Department of Biology, Davidson College

The morphology of a neuron's axonal and dendritic arbors plays a crucial role in how the neurons signal to each other and integrate information from various synaptic inputs. The development of retinal ganglion cells (RGCs) from their somas in the retina to their synaptic targets in the optic tectum is influenced by many molecular factors, and is used as a model for understanding the development of a neuron's axons and dendrites. While many factors influence the development of RGC axons, from axon initiation and pathfinding to their arborization in the optic tectum, have been identified, factors involved in the development of RGC dendrites are less well known. This study examined the effect of neural activity on Xenopus laevis RGC dendrite development in vivo. When TTX, which blocks voltage-gated Na+ channels, was used to silence retinal activity, Xenopus RGC axons developed more complex arbors (Cohen-Cory, 1999). It was therefore hypothesized that RGC dendrites would also increase their complexity in response to the inhibition of retinal neural activity. Although early light exposure does not influence Xenopus RGC dendrite development (Rigel and Lom, 2004), it is important to examine the possible contribution of spontaneous activity toward shaping the developing dendritic arbor. Intraocular injections of 100 μM TTX were used to silence action potentials in the retinae of stage 38 Xenopus tadpoles, the stage at which RGC dendrites have just begun to extend from the cell soma. At stage 43-44, when the RGC axons have reached their target in the optic tectum and dendritic arborization is well underway, rhodamine dextran was microinjected into the optic tectum to retrogradely label RGC dendritic arbors in the retina. Tadpoles were fixed at stage 45 and confocal microscopy was used to visualize RGC dendritic arbors. TTX treatment resulted in a significant increase in the average number of primary dendrites extending from each RGC, without affecting the average number of branch tips per RGC, indicating that there was an increase in the overall number of branch tips per primary dendrite. Inhibition of neural activity with TTX treatment did not affect the total length of RGC dendritic arbors. Although activity plays a role in shaping RGC dendrites, other factors may have a greater influence on their development.

**40. Zebrafish Neurogenesis is Compromised by Early Exposure to the Pesticide Malathion**

Carroll KJ & Lom B
Department of Biology, Davidson College

Malathion is an organophosphate insecticide designed to control mosquitoes, flies, boll weevils, and lice. It is an acetycholinesterase (AChE) inhibitor that disrupts nervous system function by enhancing acetylcholine (ACh) activity in the synaptic cleft. Malathion contaminates water through aerial application, runoff, and erosion and therefore can exert harmful effects on non-target aquatic organisms such as fish and amphibians. We used islet-1 GFP zebrafish to study the effects of malathion on early vertebrate neurogenesis. Islet-1 GFP zebrafish express green fluorescent protein (GFP) in a subset of neurons. In the spinal cord, GFP is first expressed by secondary motoneurons and interneurons. We treated islet-1 GFP zebrafish embryos with 2.5 mg/L of malathion, vehicle control (acetone), or dilution control for 48, 72, or 96 hours. We then examined the number and position of GFP expressing neurons in a segment of the tail just posterior to the gut using confocal microscopy. We observed significantly fewer total neurons in the embryos treated with malathion compared to both vehicle and dilution controls at 48 hours post fertilization (hpf), significantly fewer total neurons compared to the vehicle control at 72 hpf, and no difference in the number of neurons at 96 hpf relative to the dilution or vehicle controls, suggesting that this pesticide alters early motoneuron differentiation in zebrafish but that malathion's effects become less pronounced as exposure time increases. Shorter exposure durations reveal that malathion's most teratogenic effects occur during 0-24 hpf rather than 24-48 hpf, suggesting that neurogenesis in zebrafish is most reduced by malathion exposure in very early development. Taken together, these results indicate that changes in cholinergic activity can influence early neurogenesis in the spinal cord.

**41. PLCβ4 Gene Expression Profile in Mouse Brain and Liver Tissue**

Klein BM, Martin K, and Meyer-Bernstein EL
Department of Biology, College of Charleston

Animals exhibit daily rhythms that are regulated by an internal timekeeper. This timekeeper maintains and synchronizes a number of body processes including sleep/wakefulness, rest/activity, body temperature, and behavior. Individual tissues show endogenous cycles of genes and proteins but these independent cycles are only synchronized if in communication with the suprachiasmatic nucleus (SCN) of the hypothalamus. We have recently shown that a protein which is linked to circadian function in the SCN, phospholipase C β4 (PLCβ4), undergoes a circadian oscillation in both the SCN and liver tissue. Moreover, in the liver, PLCβ4 translocates from the cytoplasm to the nucleus over the course of the day. To determine if this oscillatory profile is due to rhythmic transcriptional regulation, we are currently analyzing the temporal profile of plca4 gene expression in liver obtained from animals housed in constant darkness using RT-PCR. In the brain, we are employing in situ hybridization techniques to look at light and temporal regulation of gene expression. These data are designed to gain insight into the mechanism that regulates the transcription of the plcβ4 gene in the SCN and liver of the mouse.

**42.Feeding controlled circadian rhythm of phospholipase Cβ4 and PER proteins in mouse hepatocytes.**

Chianella A, Williams D, Klein B, and Meyer-Bernstein EL
Department of Biology, College of Charleston

Bodily processes of all living things revolve around an internal timing system. In mammals, the suprachiasmatic nucleus (SCN) in the brain's hypothalamus acts as an endogenous master clock, controlling all oscillatory systems throughout the body. Other organs, including the liver, also exhibit circadian oscillations, which are synchronized by signals from the SCN. In the SCN, the clock is reset daily by light. Temporal signals in the form of hormones or food have been shown to reset the peripheral clocks, specifically the liver. Restricting the availability of food at specific times in the day will uncouple the liver's codependent circadian oscillation from that of the SCN. In the liver, a core clock protein, PERIOD (Per), has been shown to translocate between the nucleus and the cytoplasm during the circadian day, with peak nuclear staining at night. The total amount of Per protein also fluctuates throughout the day, peaking at dusk. We have recently found that the enzyme phospholipase C β4 (PLCβ4) undergoes a similar nuclear translatory oscillation and an oscillation in protein levels. In the present study, we used immunohistochemical and western blotting techniques to examine the influence of a food restriction stimulus on the temporal expression of Per and PLCβ4 in mouse hepatocytes. We compared the cellular distribution as well as total protein levels between ad libitum fed mice and mice on a restricted feeding schedule. Our preliminary results show that the timing of nuclear translocation of both PLCβ4 and Per1 in ad libitum mice is reversed compared to mice under food restriction. These results show that the nuclear translocation of Per1 and PLCβ4 is regulated by food availability. Moreover, regulation of PLCβ4 enzyme levels and cellular distribution are either directly responsive to a food stimulus or serve as an output of the circadian clock in hepatocytes.

# Further Reading

- Rohrbough *et al*. (2003) Cellular Bases of Behavioral Plasticity: Establishing and Modifying Synaptic Circuits in the *Drosophila* Genetic System. *J Neurobiol* **54**:254-271.
- The Persistent Vegetative State: Neuroethics, Communication and Evidence-based Clinical Decision Making by Jerome E. Kurent, M.D., M.P.H.
- Medical School Application Tips (Karen Eippert)
- Applying for Grants and Fellowships (Chris Korey, Ph.D.)
- Careers in Technology Transfer (Yashmin Karten, Ph.D.)
- Careers in Medical Writing (Christine Lauay, Ph.D.)
- Neurons in Action 2 (Ann Stuart, Ph.D.)

# Cellular Bases of Behavioral Plasticity: Establishing and Modifying Synaptic Circuits in the *Drosophila* Genetic System

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**ABSTRACT: Genetic malleability and amenability to behavioral assays make *Drosophila* an attractive model for dissecting the molecular mechanisms of complex behaviors, such as learning and memory. At a cellular level, *Drosophila* has contributed a wealth of information on the mechanisms regulating membrane excitability and synapse formation, function, and plasticity. Until recently, however, these studies have relied almost exclusively on analyses of the peripheral neuromuscular junction, with a smaller body of work on neurons grown in primary culture. These experimental systems are, by themselves, clearly inadequate for assessing neuronal function at the many levels necessary for an understanding of behavioral regulation. The pressing need is for access to physiologically relevant neuronal circuits as they develop and are modified throughout life. In the past few years, progress has been made in developing experimental approaches to examine functional properties of identified populations of *Drosophila* central neurons, both in cell culture and *in vivo*. This review focuses on these exciting developments, which promise to rapidly expand the frontiers of functional cellular neurobiology studies in *Drosophila*. We discuss here the technical advances that have begun to reveal the excitability and synaptic transmission properties of central neurons in flies, and discuss how these studies promise to substantially increase our understanding of neuronal mechanisms underlying behavioral plasticity.** © 2003 Wiley Periodicals, Inc. J Neurobiol 54: 254–271, 2003

***Keywords: Drosophila*; behavioral plasticity; cellular bases; synpatic circuits**

## INTRODUCTION

*Drosophila* neurobiology is poised to enter a new realm of investigation into neuronal function and plasticity. The possibility of directly assaying neuronal activity and synaptic transmission in behaviorally relevant circuits within the *Drosophila* CNS has for decades been a tantalizing prospect. The goal, of course, has been to take advantage of the wealth of precisely characterized *Drosophila* behavioral mutants as tools to unravel the cellular and molecular bases of behavior modulation. Particularly attractive in this collection are the numerous mutants with altered olfactory learning or memory. These mutants have provided key insights into the conserved molecular pathways underlying memory formation, most notably genes encoding components of $Ca^{2+}$- and cAMP-dependent signaling pathways (Davis et al., 1995; Dubnau and Tully, 1998). Such genes drive behavior by modifying the activity of neural circuits composed of individual neurons linked by chemical synaptic connections. The modulation of neuronal

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excitability, firing patterns, and efficacy of interneuronal synaptic communication within these circuits, provides a mechanistic basis for behavioral plasticity. Exploiting the *Drosophila* genetic system to its full potential as a model for behavioral plasticity demands sophisticated functional assays of central neurons and central synapses.

Despite the obvious genetic advantages of the *Drosophila* model system, the small size and inherent complexity of the fruit fly CNS has presented a formidable barrier to functional analysis of neuronal properties and central transmission relevant to behavior. By default, the vast majority of studies of synaptic transmission and activity-dependent plasticity in behavioral mutants have utilized the embryonic and larval glutamatergic neuromuscular junction (NMJ) which, in comparison to central synapses, is large, simple, and easily accessible. Although the NMJ remains an invaluable synaptic system, dissecting the cellular properties of central neurons and synapses is critical to understanding how activity in identified circuits mediates specific behaviors. How do electrical properties vary among *Drosophila* neurons? How do transmission properties vary between different chemical classes of synapses, or at different stages of development? What types of activity-dependent plasticity are present in the *Drosophila* CNS, and do different classes of synapses share common plasticity mechanisms? Surprisingly, at this late stage in the field of *Drosophila* neurobiology, these fundamental questions remain largely unaddressed. Unraveling the mechanisms of behavioral change in flies clearly requires direct investigation and thorough understanding of excitability and synaptic transmission in identified populations of neurons within the CNS.

This review focuses on recent advances in the analysis of central neuronal function in *Drosophila*. The primary interests of these studies are (1) the development of circuits that mediate behavior, particularly the acquisition of electrical and synaptic properties; (2) the excitability and synaptic transmission characteristics of mature circuits, especially the signaling properties of different chemical classes of synapses; and (3) the activity-dependent modulation of circuits that drive behavioral change, including both short-term (millisecond to minutes) and long-term (hour and longer) alterations in neuronal properties. The complementary approaches being used include both *in vitro* and *in vivo Drosophila* CNS preparations. Although *Drosophila* neuronal cultures have a long history in the investigation of neuronal excitability properties, recent studies demonstrate that they can also be successfully employed to explore the mechanisms involved in central synaptic transmission and plasticity. Moreover, the first electrophysiologic analyses of neuronal excitability and synaptic transmission in the intact *Drosophila* CNS in the last several years have refuted the dogma that central synaptic assays in this system are untenable. These recent advances represent the first steps towards the development of preparations to study the functional properties of central synaptic circuits mediating specific behaviors in the *Drosophila* genetic system.

## NEURONAL EXCITABILITY PROPERTIES AND SYNAPTIC TRANSMISSION IN *DROSOPHILA* NEURONAL CULTURE PREPARATIONS

### Backgound: Primary *Drosophila* Neuronal Culture Systems

Unlike vertebrate systems, there are no reports of functional analyses in established neuronal cell lines in *Drosophila*. However, for several decades *Drosophila* neurobiologists have used embryonic and larval primary neuronal cultures to study the genetic regulation of neuronal properties *in vitro.* Cultures of dissociated larval brain neurons were first described by C.-F. Wu and colleagues (Wu et al., 1983). These cultures are prepared by removing the central brain region and ventral ganglion from third instar larvae, dissociating the neural tissue by enzymatic digestion and mechanical trituration, and plating the cells in a serum-supplemented medium. Larval neuronal cultures have been useful in characterizing the functional properties of neuronal potassium channels, and the role of genes/signaling cascades important in potassium current regulation (Solc and Aldrich, 1988; Wright and Zhong, 1995; Delgado et al., 1998). However, sodium currents, action potentials, and synaptic transmission have yet to be demonstrated and described in these neurons, precluding analysis of the genetic regulation of these properties using these cultures. Embryonic neuronal cultures prepared from mid-gastrula stage embryos were first described by P. Seecof and colleagues (Seecof et al., 1971, 1973a). Embryonic neurons developing in culture not only express voltage-gated potassium channels, but many are electrically excitable and form functional synaptic connections. Thus, embryonic cultures have made it feasible to exploit the *Drosophila* system to explore the genetic regulation of neuronal excitability, ion channel expression, and synaptic transmission, as detailed in the following sections.

Three variations of the embryonic neuronal culture system have been developed. All are prepared by

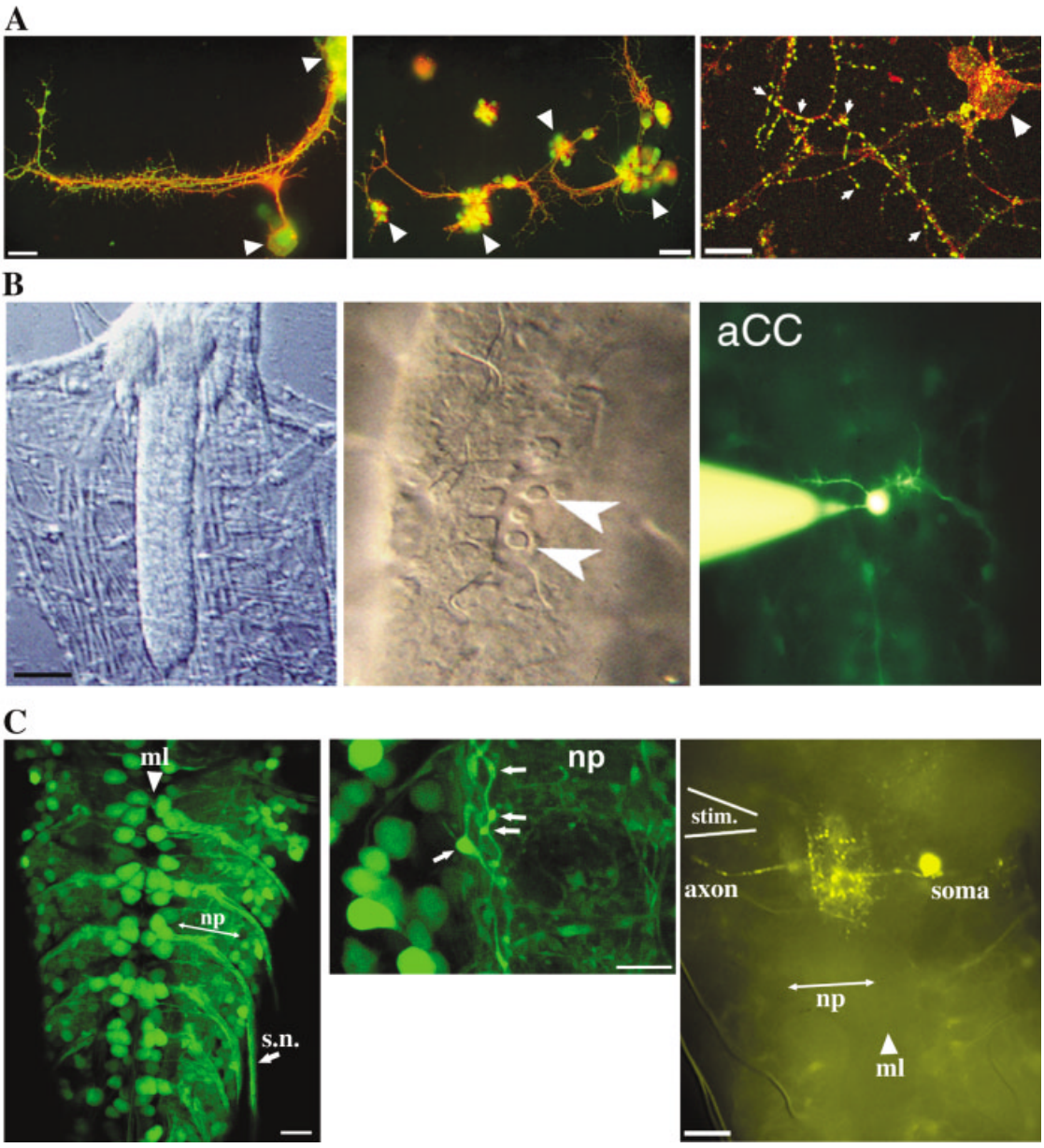


**Figure 1** Illustration of three *Drosophila* central neuronal preparations. (A) Embryonic neuronal culture system. Differentiated neurons prepared from gastrula-stage embryos, grown in traditional serum-supplemented medium. Left and center: green fluorescent protein (GFP) expression is driven in all neurons by the panneuronal elav GAL4 driver. Cultures are stained with antibodies against the neuronal-specific HRP (red). Left: two neurons with extensively interconnected neuritic processes; neuronal somae are indicated by arrowheads. Center: several clusters of neuronal somae (arrowheads), each derived from dividing neuroblasts, with fasciculated and interconnected neurites. Right: double antibody-stained embryonic neuronal culture (red = HRP; green = CSP) showing distinct, punctate localization of the synaptic vesicle associated Cysteine String Protein (CSP) along neurites and at neurite–neurite contacts. Arrowhead indicates a cluster of several somae. Scale: 8 $\mu$m (left), 20 $\mu$m (center), 10 $\mu$m (right). (B) Embryonic ventral nerve cord (VNC) preparation. Left: dorsal view of a dissected *Drosophila* embryo, showing dorsal aspect of the VNC and surrounding body wall musculature. Middle: detailed view of motor neuron somae (arrowheads) in adjacent segments, exposed by brief focal protease application. Right: an aCC motor neuron in a living preparation filled with fluorescent dye via the recording patch electrode (at left), revealing cell morphology and confirming the identity of the targeted cell. Scale: 100 $\mu$m (left), 20 $\mu$m (center and right). (C)

removing the entire cellular contents of midgastrula (stage 7) embryos and plating the dissociated cells in culture medium. In original "Seecof culture" conditions, cells are grown in a modified Schneiders medium supplemented with 20% fetal calf serum. Several cell types differentiate in these cultures, including neuroblasts that repeatedly divide and differentiate to form clonal lineages of neurons (Seecof et al., 1971, 1973a,b) [Fig. 1(A)], as well as myotubes. In the early 1990s, it was shown that blocking cell division by addition of cytochalasin-B to the serum-conditioned growth medium results in the differentiation of large (10–15 $\mu$m soma diameter) multinucleate cells with neuronal morphology, that have been termed "giant" neurons (Wu et al., 1990). In the most recent variation of embryonic culture conditions, cells are plated in a defined, serum-free, bicarbonate-buffered medium (O'Dowd, 1995; O'Dowd et al., 1995). These "defined medium" cultures are composed primarily of neurons, some with cell bodies as large as 10–15 $\mu$m in diameter [Fig. 2(A)]. Very few non-neuronal cells differentiate under these growth conditions.

## Genetic Regulation of Neuronal Excitability and Firing Properties in Cultured Embryonic Neurons

Early studies of embryonic cultures showed that extracellular stimulation of neurite bundles terminating on mutlinucleate myotubes resulted in myotube contraction, illustrating that these neurons were capable of forming functional neuromuscular synaptic connections (Seecof et al., 1973b). One disadvantage associated with these cultures is that the neurons are relatively small (~4 $\mu$m soma diameter) and fragile, and thus not amenable to traditional intracellular recording techniques using sharp microelectrodes. However, with the advent of the whole-cell recording technique, it became feasible to examine voltage-gated ion channels in these cultured embryonic neurons (Byerly and Leung, 1988; O'Dowd and Aldrich, 1988, Leung et al., 1989). Electrophysiologic characterization of specific ionic currents demonstrated that wild-type neurons expressed voltage-gated sodium, calcium, and potassium currents with similar biophysical properties to currents described in a variety of vertebrate and invertebrate neurons. Analysis of excitability mutants proved a useful strategy in exploring the functional role of putative ion channel genes, in particular those thought to encode or regulate sodium currents, such as *no action potential* (*nap*), *seizure*, *paralytic* (*para*), and *tipE* (O'Dowd and Alrich, 1988; O'Dowd et al., 1989). Altered sodium channel expression and functional gating properties were demonstrated in neurons from the *para* mutant (O'Dowd et al., 1989). These findings, in conjunction with sequence analysis of the *para* gene (Loughney et al., 1989), revealed that *para* encodes a sodium channel expressed in embryonic neurons.

In addition to traditional mutant analysis, embryonic neuronal cultures have been utilized to assess the function of genes for which no viable mutants are available. The effect of homozygous lethal mutations, including deficiencies, can be assayed in cultures prepared from single embryos obtained from heterozygous parental stocks each having one copy of the mutation or deficiency over a "balancer" chromosome. Homozygous single embryo cultures are identified by the absence of expression of reporter, or "marker," genes present on the balancer chromosomes. Initial studies employing hsp70–lacZ-marked balancers revealed that a sodium channel homolog (distinct from *para*) was not necessary for sodium channel expression in cultured embryonic neurons (Sakai et al., 1989; Germeraad et al., 1992). This approach was particularly important in the identification and functional characterization of genes encoding neuronal voltage-gated potassium channels, because there are no viable mutants in the *Shaw*, *Shal*. and *Shab* voltage-gated potassium channel subfamilies, and the *Shaker* potassium channel is not highly expressed in neurons (Solc et al., 1987; Baker and

Larval VNC preparation. Left: confocal microscope image of dorsally situated clusters of motor neurons along the midline (mL) of the VNC in an elav GFP larval preparation. Double arrows indicate the lateral margins of the central neuropil (np). Large arrow indicates a segmental nerve (s.n.) exiting the VNC. Middle: detailed confocal image of cholinergic neurons and central cholinergic processes within the neuropil, visualized by GFP expression throughout the cholinergic nervous system (Cha GAL4 UAS GFP larva). Arrows indicate putative synaptic varicosities along cholinergic processes. Right: morphology of a recorded motor neuron, visualized by lucifer yellow introduced via the recording patch pipette. The image was taken following recording and brief fixation. A contralateral motor axon and dendritic arborization are clearly visualized. Position of a stimulating electrode (stim.) placed in the neuropil during the experiment is depicted (see also Fig. 7). Arrowhead indicates the VNC midline. Scale: 20 $\mu$m (left, right), 10 $\mu$m (center).

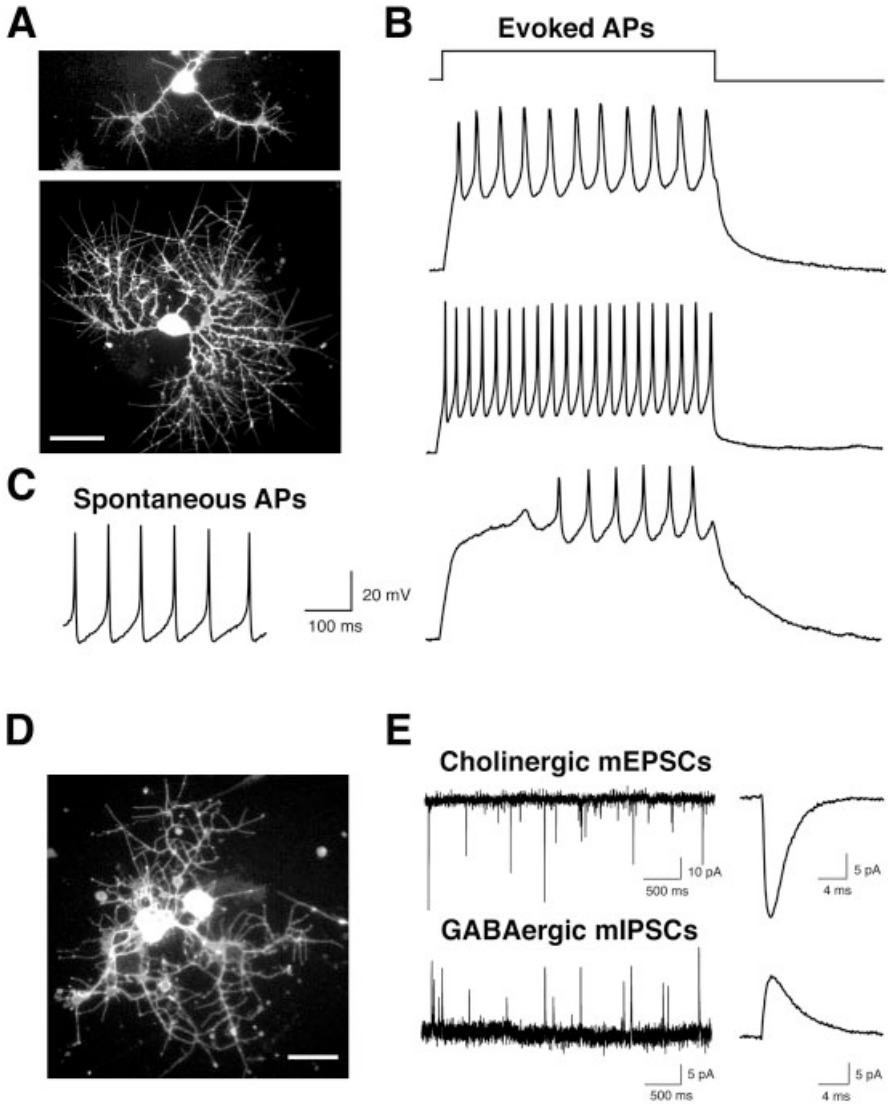


**Figure 2** Embryonic neurons grown in defined medium are electrically excitable and form functional interneuronal synaptic connections. (A) Fluorescent images of two isolated embryonic *Drosophila* neurons grown in DDM1 defined medium (O'Dowd, 1995). Cells were fixed and stained with fluorescently conjugated anti-HRP antibody. Scale: 20 $\mu$m. (B) Whole-cell current clamp recordings illustrate three of the distinct firing patterns observed in cultured neurons in response to sustained step depolarizations. (C) Voltage recording in the absence of an applied stimulus, illustrating typical action potentials seen in spontaneously active neurons. (D) Fluorescent photomicrograph of a small cluster of embryonic neurons and their extensively overlapping neuritic processes. Scale: 20 $\mu$m. (E) Synaptic currents recorded from cultured neurons exhibiting morphologic contact with the neurites of one or more neighboring neurons. Spontaneous cholinergic miniature excitatory postsynaptic currents (mEPSCs) are inward at $-75$ mV, and the fast kinetic features of these currents are illustrated in the high-resolution ensemble average trace shown to the right. Spontaneous GABAergic miniature inhibitory postsynaptic currents (mIPSCsO are recorded as outward currents at 0 mV, and kinetic properties are illustrated in the accompanying ensemble average trace.

Salkoff, 1990). Deficiency analysis revealed that the wide range of whole-cell potassium currents observed in embryonic *Drosophila* neurons arises from the channels encoded by the *Shal*, *Shaw*, and *Shab* genes (Tsunoda and Salkoff, 1995). In these early studies, use of the hsp70–lacZ marker system necessitated that culture genotype be identified only following a postphysiology heat shock to induce $\beta$-galactosidase

expression, which was visualized following exposure to its enzyme substrate, X-gal. Recent construction of balancer chromosomes containing green fluorescent protein (GFP) coding sequences, under the control of constituitive promoters such as ubiquitin and actin, now make it possible to identify the genotype of living single embryo cultures prior to recording.

A detailed characterization of neuronal firing properties is a fundamental prerequisite for defining genes involved in plasticity in the nervous system. Although it is possible to record voltage-gated ionic currents from neurons in the serum-supplemented embryonic cultures, there are no reports describing firing properties, or synaptic transmission in these neurons. However, in the early 1990s it was found that "giant" multinucleate neurons in cytochalasin B-treated cultures are electrically excitable (Saito and Wu, 1991). Analysis of neurons from two mutants, *slowpoke* (*slo*) (Saito and Wu, 1991) and *Hyperkinetic* (*Hk*) (Yao and Wu, 1999), demonstrated that these genes, encoding a calcium-activated potassium channel and a potassium channel $\beta$-subunit, respectively, are involved in regulation of "giant" neuron excitability. In addition, altered firing properties were demonstrated in "giant" neurons from two behavioral learning/memory mutants; *dunce* (cAMP phosphodiesterase) and *rutabaga* ($Ca^{2+}$-dependent adenylate cyclase). These findings suggest that $Ca^{2+}$- and cAMP-dependent regulation of neuronal excitability may be involved in the plasticity necessary for aspects of learning and memory (Kim and Wu, 1996; Zhao and Wu, 1997; Yao and Wu, 2001).

Neurons in defined medium cultures [Fig. 2(A)] are also electrically excitable, exhibiting a variety of distinct evoked firing phenotypes [Fig. 2(B)]. In addition, some of these neurons fire action potentials spontaneously [Fig. 2(C)]. A recent analysis of *tipE* mutant neurons in defined medium cultures has revealed a role for the *tipE* gene in regulating action potential firing (Hodges et al., 2002). Neurons in *tipE* mutant cultures have a decreased capability for firing repetitive action potentials. The mutant firing phenotype is rescued following expression of a wild-type *tipE* transgene in the mutant background, confirming the specific requirement for the *tipE* gene. Even more interesting was the finding that in mutant neurons with already established firing properties, transgenic *tipE*+ expression rescues the mutant firing phenotype within 24 h of induction. These observations suggest that *tipE* plays a role not only in establishment of neuronal firing phenotype, but also in modulation of firing properties in differentiated neurons. Because alterations in neuronal excitability are likely to play an important role in plasticity in the nervous system, the physiologic analysis of mutant neurons, both in defined medium and in "giant" neuron cultures, should continue to provide insight into genes that are involved in this process.

The *Drosophila* embryonic cultures are unique in that the neurons arise from precursors that undergo mitosis as well as differentiation *in vitro* (nuclear division continues in the absence of cytoplasmic divisions in cytochalasin-B–treated cultures). With the exception of recent advances in stem cell-derived neural cultures (Song et al., 2002a, 2002b), primary neuronal cultures from other species are typically prepared from postmitotic neurons that have already undergone a significant period of development and differentiation *in vivo*. In many cases, neuronal differentiation is considerably advanced at the time that cells are removed from their established context and placed in culture. Thus, at least in early subsequent stages *in vitro*, the processes the experimenter is examining are more likely to reflect regeneration, rather than *de novo* development. *Drosophila* midgastrula stage cultures therefore provide a unique opportunity to explore environmental factors that influence very early developmental processes of neuronal determination. It is an obvious concern whether a heterogenous population of neurons developing completely *in vitro* acquires functional properties appropriate for their lineage. Recent studies have shown that the progeny of single identified neuronal precursor cells grown in culture express some of the molecular markers and functional properties expected for that lineage (Luer and Technau, 1992; Schmidt et al., 2000). Such studies may identify aspects of early neuronal differentiation that are cell autonomous while highlighting other phenotypes that are influenced by environment.

## Studies of Fast Neuronal Synaptic Transmission and Synaptic Plasticity Mechanisms in Cultured Embryonic Neurons

A complete study of *Drosophila* neuronal plasticity clearly requires the ability to examine, as well as genetically perturb, neuronal synaptic transmission. Until recently, work on neurons in defined medium and "giant" cultured neurons had focused primarily on the excitable properties of single, isolated cells. However, when cultured at appropriate densities, neurons differentiating in both culture systems form numerous morphologic contacts with neighboring neurons, in some cases producing extensively interconnected networks of cells [Fig. 1(A)]. These cells express synaptic vesicle molecular markers localized to varicosities that resemble synaptic boutons [Fig. 1(A)], and also

express functional acetylcholine (ACh) receptor channels (J. Rohrbough and K. Broadie, unpublished data). Spontaneous quantal ACh secretion from growth cones of isolated "giant" neurons is readily detectable when using nicotinic ACh receptor (nAChR)-rich *Xenopus* myoballs as a bioassay (Yao et al., 2000). Surprisingly, however, functional synaptic transmission between these neurons has not been well established. Demonstration of transmitter release, coupled with a recent illustration of spontaneous autaptic synaptic currents in a single "giant" neuron, suggests that studies of synaptic transmission in these cells will be possible (Yao et al., 2000).

More promising still are the results obtained from morphologically interconnected neurons grown in the defined medium [Fig. 2(D)]. Two recent reports have demonstrated fast synaptic transmission between embryonic neurons grown in these conditions, and have begun to examine genetic regulation of neuronal synaptic transmission (Lee and O'Dowd, 1999, 2000). Pharmacologic analyses reveal that the majority of fast excitatory synaptic currents are cholinergic and mediated by nAChRs (Lee and O'Dowd, 1999) [Fig. 2(E)]. Fast inhibitory synaptic currents, mediated by picrotoxin-sensitive GABA-gated chloride receptor channels (Lee and O'Dowd, in preparation), are also observed in a subset of these neurons [Fig. 2(E)]. Analysis of synaptic currents in cultures from two different alleles of the *dunce* learning mutant, which have elevated cAMP levels due to disruption of a phosphodiesterase gene (Byers et al., 1981), demonstrated a significant increase in frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs). The *dunce* gene, and thus cAMP signaling, are therefore implicated in regulation of transmission at these cholinergic synapses. Interestingly, although it has been well documented that *dunce* also regulates evoked transmission and activity-dependent plasticity at the glutamatergic NMJ synapse, increased evoked transmitter release at the *dunce* mutant NMJ appears to occur without a notable increase in mEPSC incidence (Zhong and Wu, 1991). This suggests that a single gene mutation can result in distinct synapse-specific defects, and is an example highlighting the necessity of continuing studies at *Drosophila* central synapses to define functional changes that contribute to central synaptic plasticity. Comparison of these results with data from the well-defined NMJ will be important in identifying both general and specific mechanisms that regulate synaptic transmission.

In addition to genetic manipulations, embryonic cultures readily permit pharmacologic perturbations to investigate signaling mechanisms involved in regulation of synaptic transmission and plasticity. For example, neuronal cultures exposed to a membrane-permeable cAMP analog, dibutyrl (db)-cAMP, exhibit elevated mEPSC frequency similar to *dunce* mutants. This result represents a pharmacologic phenocopy of *dunce*, demonstrating that elevated cAMP levels are sufficient to upregulate transmission at cholinergic synapses (Lee and O'Dowd, 2000). Studies in *Aplysia* indicate that the persistence of cAMP signaling-dependent plasticity at sensorimotor synapses is related to the temporal pattern of the stimulus. Repetitive, spaced applications of serotonin to activate cAMP signaling induce a longer lasting increase in synaptic strength than a single pulse (Glanzman, 1994). In *Drosophila* cultured neurons, brief (4-h) db-cAMP exposure is sufficient to induce a protein synthesis-dependent increase in mEPSC frequency. Four spaced 1-h db-cAMP treatments, however, produce a more persistent increase in mEPSC frequency than a single 4-h exposure (Lee and O'Dowd, 2000). These data suggest that interneuronal excitatory cholinergic synapses in the *Drosophila* CNS, like central excitatory glutamatergic synapses in other species, are sites of cAMP-dependent modulation. An inability in the *dunce* mutants to produce persistent increases in mEPSC frequency over already-elevated basal levels, is consistent with a role for cAMP-dependent plasticity at cholinergic synapses. These studies provide some of the first direct supporting evidence at the cellular level for synaptic modulation mechanisms contributing to behavioral learning and memory formation in *Drosophila*.

## Neuronal Excitability and Synaptic Transmission in Cultured Adult Mushroom Body Neurons: Preliminary Studies

Because the overwhelming majority of *Drosophila* behavioral studies are performed on adult flies, an important goal for *in vitro* plasticity studies is to identify and record from neurons known to mediate specific behaviors, such as learning and memory, or responses to drugs of abuse. Adult CNS neurons are born and differentiate during late larval and early/mid pupal stages, and can be dissociated from the brains of these animals and cultured. Using a lacZ reporter in cultures prepared from third instar larval brains, it has been possible to record from neurons of the mushroom body (MB) (Wright and Zhong, 1995; Delgado et al., 1998), a brain area with a well-defined role in olfactory learning and memory (Davis, 2001). These cultured larval MB neurons express a variety of voltage-gated potassium currents that have been exten-

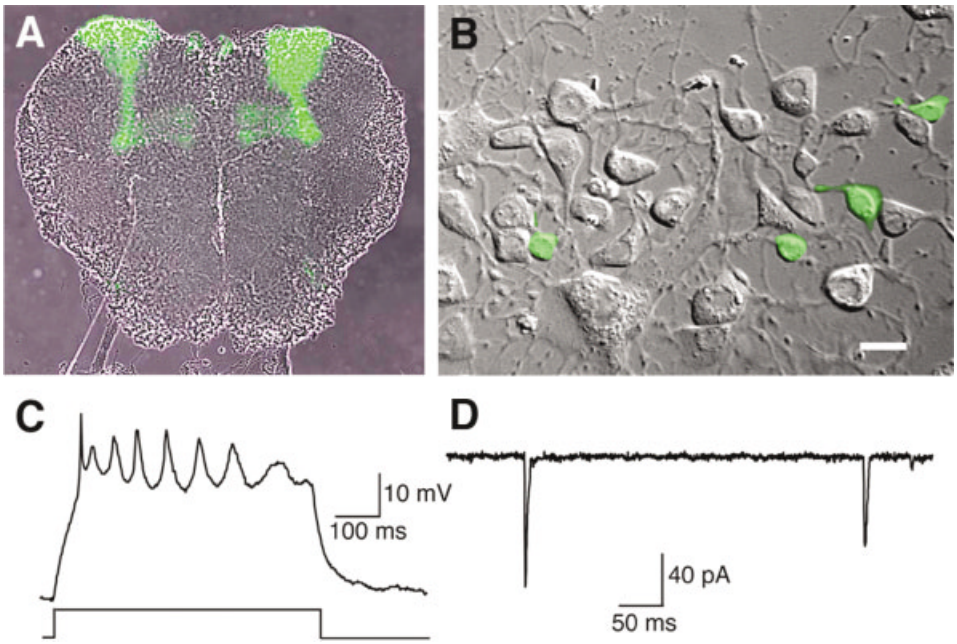


**Figure 3** Electrically excitable and synaptically connected neurons in pupal brain cultures. (A) Image of the central brain region removed from a 107-GAL4; UAS-GFP pupa (72 h after pupariation). Fluorescent and bright-field images are superimposed. High levels of GFP expression clearly outline the mushroom body regions in the whole brain tissue. (B) Pupal cells grown in dissociated culture for 9 days exhibit extensive process outgrowth. A fluorescent image is superimposed on a Nomarski photomicrograph. Mushroom body neurons in culture are identified as those that are GFP positive. Scale: 10 $\mu$m. (C) Whole-cell current clamp recording of an action potential train in a cultured pupal neuron evoked by a sustained step depolarization. (D) Spontaneous EPSCs recorded from a cultured pupal neuron at a holding potential of $-75$ mV.

sively studied (Wright and Zhong, 1995; Delgado et al., 1998). However, there are no reports describing sodium currents, sodium-dependent action potentials, or synaptic transmission in these neurons. This is surprising, as a variety of voltage-gated currents and responses to multiple neurotransmitters have been reported in cultured MB neurons from other insects including, honeybees (Bicker, 1996; Cayre et al., 1998, 1999). Therefore, until conditions are identified that promote maturation of these neuronal properties in the *Drosophila* larval brain neuron cultures, the opportunity for investigating genes involved in adult synaptic transmission and plasticity is limited.

On a promising note, recent studies demonstrate that neurons can be harvested from the central brain regions of late pupae (P9 through preeclosion adults), and cultured using a protocol similar to that previously developed for early pupal brain neurons (Kraft et al., 1998). Late pupal neurons regenerate processes and form extensively overlapping neuritic networks in culture [Fig. 3(A) and 3(B)]. These neurons express voltage-gated sodium, calcium, and potassium currents, and a subset are electrically excitable [Figu.3(C)]. Functional synaptic connections [Fig. 3(D)] are also formed between neurons (H. Su and D.K. O'Dowd, in preparation). Crosses of GAL4 enhancer-trap lines to neuron-specific UAS–GFP lines can be used to identify and record from specific neuronal subpopulations in these cultures, including MB neurons [Fig. 3(B)]. Such results demonstrate promise for synaptic transmission and modulation studies in identified adult neurons on the near horizon.

In summary, analyses of excitability and synaptic transmission properties in cultured *Drosophila* neurons promises to contribute significantly to illuminating the cellular bases of behavioral plasticity. Although cultured neurons are obviously differentiating or regenerating in conditions very different from those *in vivo*, emerging data from neuronal recordings in the intact *Drosophila* embryonic and larval ventral nerve cord (see below), and implementation of the UAS–GAL4 system to target GFP expression in specific neurons, now allows direct comparisons of electrophysiological properties of neurons *in vivo* and *in vitro*. These approaches open the door for experiments aimed at defining cell autonomous properties,

and perhaps more importantly, at identifying factors that influence non cell autonomous neuronal properties.

## NEURONAL EXCITABILITY AND SYNAPTIC DEVELOPMENT, FUNCTION, AND PLASTICITY *IN VIVO*

An essential objective in *Drosophila* neurobiology is to examine the functional properties of defined neuronal circuits that drive known behaviors. Attaining this goal ultimately requires that detailed recordings be made from neurons in the adult brain, to correlate most directly with adult behavioral studies. In terms of cell numbers and anatomical complexity, however, the adult brain is far less tractable than the relatively simple nervous system that develops in the embryo to drive larval behavior. *Drosophila* embryonic and larval NMJ physiologic preparations have been extensively used to examine electrical excitability properties (primarily in muscle fibers) and many detailed features of synapse formation, transmission, and plasticity at a peripheral glutamatergic synapse. However, the question of how central synapses form, function, and regulate behavior in this model system has remained largely unexplored. In just the last few years, studies have for the first time begun to directly access central neurons in the already familiar embryonic and larval preparations to examine the developing motor system. This work is aimed at addressing several related questions: (1) what are the electrical properties or signatures of identified central neurons *in vivo*, and how are these properties acquired and modulated? (2) How is central synaptic connectivity established, and what pre- and postsynaptic interactions are involved? (3) What classes of synaptic transmission are present in identified central neurons, and how are functional transmission and plasticity mechanisms regulated? Addressing these questions in an amenable CNS model preparation is necessary to link information from studies performed in culture and at the peripheral neuromuscular synapse, and provides an initial step toward future studies coupling adult CNS physiology and behavioral analyses.

### Development of Neuronal Excitability in Identified Embryonic Central Neurons

The regulatory processes governing the establishment of neuronal electrical properties *in vivo* remain largely unknown. Factors that likely determine or influence neuronal characteristics *in vivo* can be broadly divided into those that are extrinsic, and those that are intrinsic or autonomous to individual neurons. For example, the identity of an individual neuron is likely to be determined both as a result of its lineage within a particular neuroblast clone, and from interactions with neighboring cells during embryogenesis (Doe and Goodman, 1985; Kuwada and Goodman, 1985; Doe and Skeath, 1996). Differences in lineage are often reflected by a differential expression of specific transcription factors (Goridis and Brunet, 1999). With this in mind, it will be important to establish whether expression of a particular transcription factor(s) is decisive in establishing the specific excitable properties of identified *Drosophila* neurons. Equally important, however, is the extent to which the formation of synaptic connections, or exposure to presynaptic neurotransmitters or other trophic molecules, are able to modify the acquisition of electrical properties. Final neuronal identity, including the mix of ion channels expressed, will likely be dictated by a combination of such extrinsic cues as well as intrinsic cell-specific programs. Because cell autonomous properties, extrinsic environmental factors, and normal neuronal circuitry are maintained in the intact developing animal, *in situ* CNS studies provide the most direct approach to investigating behaviorally relevant neuronal properties.

Despite the dauntingly small size of the CNS at all stages of development, and the seeming inaccessibility of central neurons, *Drosophila* nevertheless provides many advantages for *in vivo* analysis of functional neuronal properties during development. Many neurons, particularly motor neurons, can be individually identified based on their clonal lineage, soma position, axonal projections, and dendritic morphology within the nerve cord (Landgraf et al., 1997). Moreover, using the GAL4/UAS system, targeted GFP expression can be used to visualize individual neuronal subpopulations (see below), or to target transgene expression to specific identified neurons (Baines et al., 1999), minimizing complications that often arise from a more widespread expression in other cell types. Initial studies in the embryo have concentrated on five neighboring motor neurons, aCC and RP1-4, and the interneuron pCC, which have cell bodies located in a dorsal position within the embryonic and larval ventral nerve cord (VNC) [Fig. 1(B)]. The cell bodies of these identified neurons are accessible to patch-clamp recording electrodes following brief focal treatment of the overlying neurolemma with protease (Baines and Bate, 1998; Baines et al., 1999).

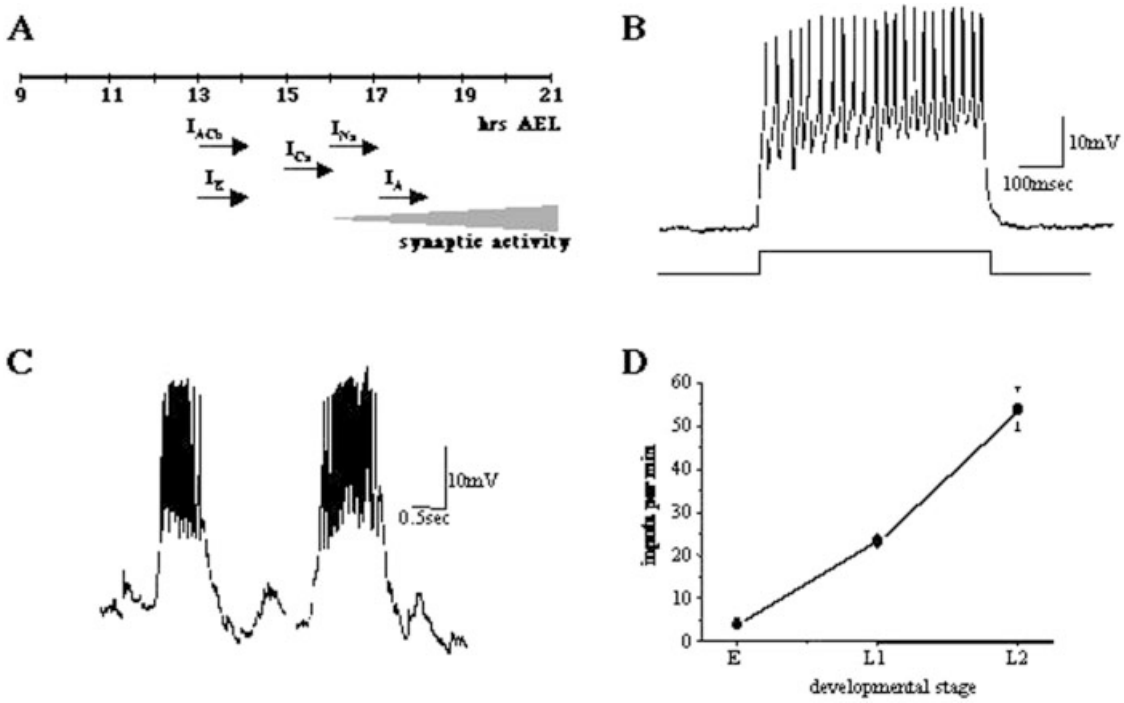


**Figure 4** Development of excitable properties and synaptic transmission in identified neurons in the *Drosophila* CNS. (A) Summary of the electrophysiologic development of the identified motor neurons aCC and RP2 during embryogenesis. Arrows show the onset of each respective current. Time axis shows hours after egg laying (AEL), with full embryonic development taking 21 h. Synaptic activity is first evident at about 16 h in the form of fast spontaneous EPSCs, followed within several hours by rhythmic patterned activity (C); the frequency and magnitude of activity increases as development proceeds (Baines and Bate, 1998; Baines et al., 2000). (B) Injection of constant current (10 pA, 500 ms) into the soma of either aCC or RP2 (RP2 shown) is sufficient to evoke repetitive action potentials. (C) Whole-cell current-clamp recordings from either aCC or RP2 (aCC shown), at the end of embryogenesis (19 h and older), or in larvae, reveal excitatory synaptic inputs that are sufficient to evoke action potentials. These excitatory responses occur rhythmically, are relatively long-lived (0.5–2 s), and entirely dependent on cholinergic transmission (Baines et al., 2001). (D) Synaptic depolarizations sufficient to evoke action potentials in aCC/RP2 are first evident in late stage 17 embryos just prior to hatching (labeled E) and increase in frequency during larval development (labeled L1, first instar and L2, second instar). Values are mean $\pm$ S.E.M., $n \leq 10$.

Electrophysiologic analyses of these neurons during late embryogenesis reveals a sequential appearance of individual voltage-gated ionic currents, including several distinct outward $K^+$ conductances, and inward $Ca^{2+}$ and $Na^+$ conductances (Baines and Bate, 1998; Baines et al., 2001) [Figs. 4(A) and 5(A). These cells manifest the ability to fire repetitive action potentials (Baines et al., 2001) [Fig. 4(B)], and exhibit agonist-gated responses and synaptically mediated activity [Fig. 4(C)–(D); see below]. This embryonic central nervous system preparation has established the first developmental framework for the onset and development of functional neuronal properties, including synaptic transmission, and forms the basis for the mature neuronal circuits mediating behavior.

## Development of Central Synaptic Connections and Functional Cholinergic Transmission

Almost nothing is known in *Drosophila* about the mechanisms by which central synapses are formed in behavioral networks, or how their relative functional strengths may be controlled. Pre- and postsynaptic elements could form relatively independently of each other or, alternatively, connectivity might require extensive interactions between synaptic partners. In the *Drosophila* embryonic CNS, the appearance and maturation of electrically excitable currents is closely paralleled by the appearance and maturation of neurotransmitter responses and endogenous synaptically mediated activity (Baines and Bate, 1998) [Fig. 4(A)].

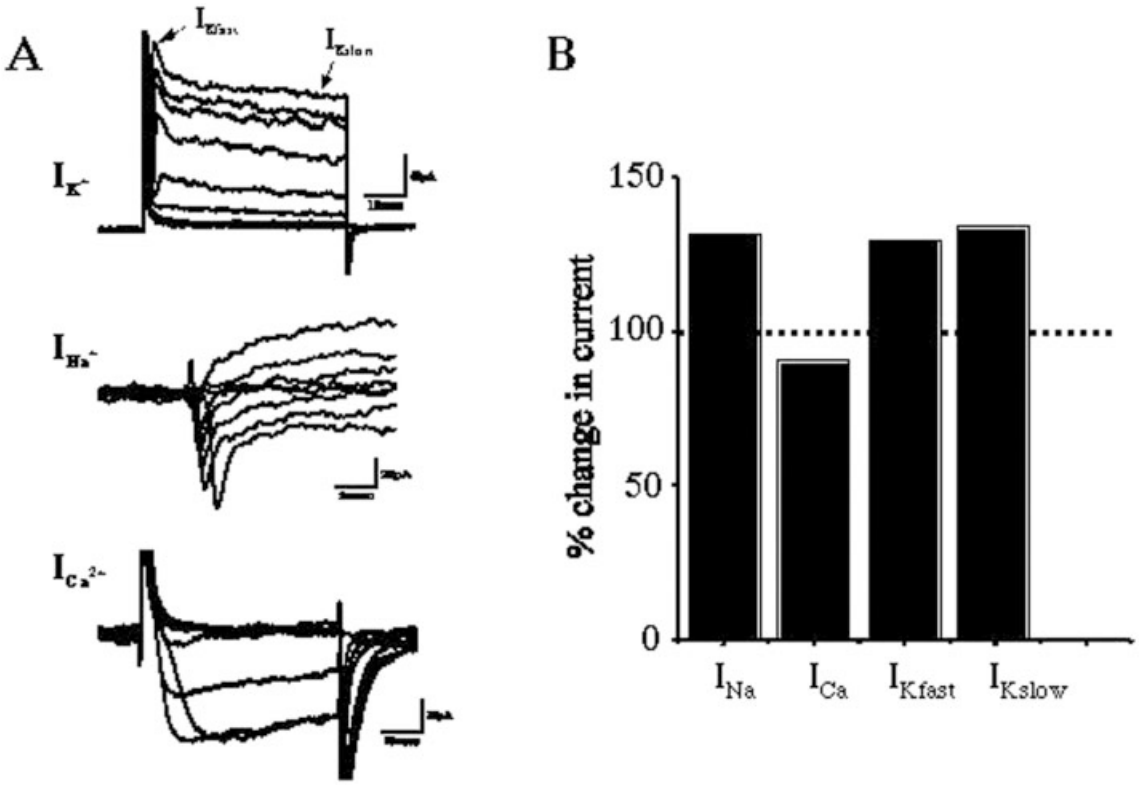


**Figure 5** The absence of synaptic input alters the developmental acquisition of electrical properties in identified motor neurons. (A) Whole-cell current traces showing typical examples of outward $K^+$- and inward $Na^+$- and $Ca^{2+}$ voltage-gated ion currents in aCC and RP2 motor neurons (currents are similar in both neurons). (B) Comparison of voltage-gated currents in aCC and RP2 in embryos that have developed in the absence of evoked neurotransmitter release, to those in control embryos with normal synaptic transmission. Bars show the average change in these currents relative to control values (indicated by dashed line set to 100%). A requirement for synaptic input in setting the magnitude of both inward $Na^+$ and outward $K^+$ currents in these neurons is clearly demonstrated.

Closely correlated with functional synaptic activity is the initial appearance and subsequent proliferation of presynaptic terminals on these neurons, which can be visualized using cell-specific labeling coupled to electron microscopy. The inputs onto motor neurons that drive locomotory firing patterns are predominantly excitatory and cholinergic (Baines et al., 1999, 2001; Rohrbough and Broadie, 2002). All motor neurons first become responsive to ACh in mid-to-late embryogenesis (13–16 h of development; hatching occurs at ~21 h) (Baines and Bate, 1998), and by hatching most, if not all, also respond to the inhibitory transmitter GABA (J. Rohrbough and K. Broadie, unpublished data). By 16–19 h, in parallel with the onset of locomotory movement, synaptic input to motor neurons is recorded in the form of both fast AP-mediated spontaneous excitatory synaptic currents (sEPSCs), and periodic, sustained (up to 1-s duration) episodes of excitatory activity occurring several times or more per minute (Baines et al., 1999, 2001) [Fig. 4(C) and (D)]. This latter "rhythmic" excitatory activity in motor neurons requires cholinergic synaptic input for its generation, because it is eliminated either by conditionally blocking ACh synthesis in cholinergic neurons, or by general blockade of spontaneous and evoked neuronal synaptic vesicle release (Baines et al., 2001). Strong, sustained episodes of cholinergic presynaptic input lead to the activation of voltage-gated currents and the generation large excitatory potentials accompanied by a burst of motor neuron AP firing [Fig. 4(C)]. The motor output of this centrally generated activity is recorded at the NMJ as patterned bursts of high-frequency excitatory (glutamatergic) transmission that drives the peristaltic muscle contraction underlying coordinated locomotion (Broadie and Bate, 1993; Baines et al., 2001; Cattaert and Birman, 2001).

## Synaptic Transmission Is Necessary for Shaping Electrical Properties of Embryonic Neurons

Central neurons developing in the absence of synaptic transmission show no apparent changes in synaptic connectivity within the CNS (Baines et al., 2001). Universal blockade of neuronal transmission can be effected by pan-neuronal expression of tetanus toxin light chain (TeTxLC), which cleaves the synaptic vesicle (SV)-associated protein n-Synaptobrevin, abolishing evoked SV release (Broadie et al., 1995; Sweeney et al., 1995). Although the possibility that central synapses might form in inappropriate areas under these conditions cannot be ruled out, it seems likely that, as at the NMJ, there is no absolute requirement for synaptic activity for the correct establishment of embryonic neuronal connectivity (Broadie et al., 1995). However, in the absence of synaptic transmission, motor neurons develop abnormal electrical properties, including significantly greater voltage-activated $Na^+$ and $K^+$ currents (Baines et al., 2001) (Fig. 5). The altered currents appear to be due at least in part to upregulated gene expression of both *para* ($I_{Na}$) and *slowpoke* ($I_{K(Ca)}$) (R. Bohm and R.A. Baines, unpublished data). The sum effect of the alterations in membrane currents appears to be increased neuronal excitability (Baines et al., 2001). This result clearly demonstrates a requirement for synaptic communication to shape the electrical properties of at least some neuronal types in the developing CNS.

Similar mechanisms have also been demonstrated in crustacean stomatogastric (STG) neurons (Turrigiano et al., 1994) and in cultured mammalian cortical neurons (Turrigiano et al., 1998; Desai et al., 1999). When STG neurons are acutely isolated from synaptic inputs, the normal *in vivo* bursting pattern of firing is lost, and is replaced with tonic firing. After several days of isolation in culture, these neurons regain the ability to fire in bursts; however, this bursting behavior can be reversed by as little as 1 h of rhythmically patterned stimulation (Turrigiano et al., 1994). Rat cortical neurons also respond to changing levels of synaptic excitation by altering both their intrinsic excitability, and the strength of their response to presynaptic excitatory neurotransmitter release. Intrinsic excitability is altered through modification of membrane conductances, while glutamatergic transmission strength is postsynaptically modulated by changes in synaptic AMPA receptor density (Turrigiano et al., 1998; Desai et al.. 1999). Through these mechanisms, mammalian cortical neurons are proposed to bidirectionally optimize their responsivity to changing levels of synaptic input, increasing or decreasing their intrinsic excitability when faced with weaker or stronger synaptic input, respectively.

Although *Drosophila* central synapse formation in general is unaffected by the absence of synaptic transmission throughout the nervous system, eliminating evoked SV release selectively in the aCC and RP2 motor neurons surprisingly alters synapse formation specifically onto these two neurons (Baines et al., 1999). Ultrastructural analysis reveals a significantly reduced number of presynaptic input sites on the tetanus toxin-expressing motor neurons. These structural changes are accompanied by a reduced incidence of synaptically mediated activity in aCC and RP2 (Baines et al., 1999). Thus, eliminating transmitter release in specific postsynaptic motor neurons also selectively impairs synapse formation and functional transmission in their presynaptic neuronal partners. These results are consistent with a model in which the synaptic output of the postsynaptic motor neuron contributes to the process of synaptogenesis presynaptically, and regulates the incorporation of developing neurons into functional networks. The precise mechanism responsible for this effect remains to be determined. Interestingly, however, the reduction in functional and morphologic synaptic input following selective motor neuron transmission blockade is phenocopied by selectively increasing aCC and RP2 expression of Fasciclin II, a homolog of Neural Cell Adhesion Molecule (NCAM) (R.A. Baines, unpublished data). These results reveal a dichotomy between the effect of global, pan-neuronal blockade of transmission, versus targeted blockade in select neurons. One possible explanation is that circuit formation in the *Drosophila* CNS is sensitively regulated by activity-dependent competition between neurons. Thus, cell-specific disruption of synaptic activity would be predicted to alter synaptic development in the affected neurons more decisively than global disruption of activity affecting all neurons equally. Such competitive mechanisms in the mammalian CNS are fundamental to the processes of synaptic retraction and consolidation. Two well-characterized examples are the activity-dependent refinement of retinotectal projections, and the competitive sorting and selective elimination of motor terminals on muscle fibres (Goodman and Shatz, 1993; Hall and Sanes, 1993; Katz and Shatz, 1996; Colman et al., 1997).

## Electrical Signatures of Identified Larval Motor Neurons *In Vivo*

Recordings from identified neurons in the larval VNC have recently begun to provide the first detailed de-

scriptions of intrinsically regulated firing patterns in the *Drosophila* CNS (J. Choi, D. Park, and L. Griffith, unpublished data and personal communication). With the aid of a motor neuron-specific GAL4 line and intracellular dye labeling, five prominent dorsal motor neurons, all of which form large "type I" glutamatergic NMJs on identified muscles, can be individually targeted. Each neuron within this population exhibits a consistent set of electrical properties, and a distinct repetitive action potential firing signature. A single neuron in this dorsal group, MNISN-Is, forms morphologically smaller type I ("Is") synaptic boutons on multiple muscle targets, and displays a characteristic delayed AP firing phenotype. This delayed firing is sensitive to membrane voltage and to the $K^+$ channel blocker 4-AP, which selectively blocks fast transient A-type $K^+$ currents. Paired recordings from both presynaptic neuron and postsynaptic muscle have made for one neuron-muscle pair (MN 6/7b and muscle 6), showing that each neuronal AP results in functional transmission at the NMJ. These results demonstrate precise neuronal soma exposure, reliable neuronal identification by GFP, and high-resolution neuronal recordings, representing significant technical steps toward directly addressing functional neuronal properties in the *Drosophila* CNS.

## Endogenous and Evoked Cholinergic Transmission in Larvae

In addition to repetitive action potential firing properties, motor neurons in the mature larval VNC respond to multiple neurotransmitter agonists. Motor neurons are strongly excited by ACh, and also respond more weakly to GABA and glutamate, both of which elicit inhibitory responses mediated primarily by picrotoxin-sensitive $Cl^-$ currents (Rohrbough and Broadie, 2002). Cholinergic neurons are located centrally throughout the lateral VNC and brain lobes. The VNC central neuropil is densely packed with cholinergic axonal fibers with presynaptic-like varicosities, which overlap spatially with the dendritic arbors of motor neurons (Rohrbough et al., 2000; Rohrbough and Broadie, 2002) [Fig. 1(C)]. Although the principle inputs to the motor neurons are thus presumed to be primarily from cholinergic interneurons, neither specific presynaptic partner(s), patterns of central presynaptic projections, or number and distribution of either cholinergic or noncholinergic synapses are currently known.

Larval motor neurons exhibit endogenous forms of synaptic activity analogous to that present in the embryo, including fast sEPSCs, and sustained excitatory "rhythmic" currents and potentials (Rohrbough and Broadie, 2002) (Fig. 6). Both fast and sustained types of endogenous activity require external calcium, and are reversibly blocked by the nicotinic ACh receptor antagonist curare, indicating that cholinergic synaptic input drives the observed motor neuron activity. Significantly, the ability to experimentally drive evoked excitatory synaptic transmission has been demonstrated at *Drosophila* central synapses for the first time in this preparation (Rohrbough and Broadie, 2002) [Figs. 6(B)–(C), Fig. 7). Electrical stimulation of the CNS triggers the generation of sustained responses with the same features and pharmacology as the endogenous forms of activity [Fig. 6(B)], indicating they share the same cholinergic synaptic basis. Furthermore, focal electrical stimulation of defined regions of the neuropil adjacent to the motor neuron dendritic regions excites fast, all-or-none EPSCs (Fig. 7), indicating that transmission at single excitatory synaptic inputs can be experimentally driven, a crucial prerequisite for on-going detailed functional synaptic plasticity assays.

Altered central transmission properties have been demonstrated in the larval CNS for two classical behavioral mutants, the temperature-sensitive paralytic mutant *shibire* (encoding Dynamin), and the learning mutant, *dunce* (encoding a cAMP-specific phosphodiesterase). In *shibire* mutants, a demonstrated block of SV endocytosis at restrictive temperature (Ikeda et al., 1976) leads to gradual SV depletion and loss of evoked transmission at the NMJ (Delgado et al., 2000; Kawasaki et al., 2000). In central motor neuron recordings, both spontaneous and evoked central synaptic transmission are also reduced under these conditions (Rohrbough and Broadie, 2002). This result demonstrates a conserved mechanism of sustained transmission at central cholinergic and peripheral glutamatergic synapses. As in recordings from cultured *dunce* neurons (above), *dunce* mutant motor neurons *in situ* also exhibit significantly elevated sEPSC frequency, in parallel with a modest increase in evoked EPSC amplitude (Rohrbough and Broadie, 2002). The elevated *dunce* SV fusion frequency, which is not observed at the NMJ (Zhong and Wu. 1991), thus appears specific to central synapses, demonstrating that there may be important differences between cAMP-mediated forms of presynaptic plasticity at different synapse types. Although evoked transmission assays in this CNS preparation require refinement, these results represent the most significant cellular characterization of central transmission in *Drosophila*, and provide a basis for functional plasticity studies in behavioral mutants *in vivo*.

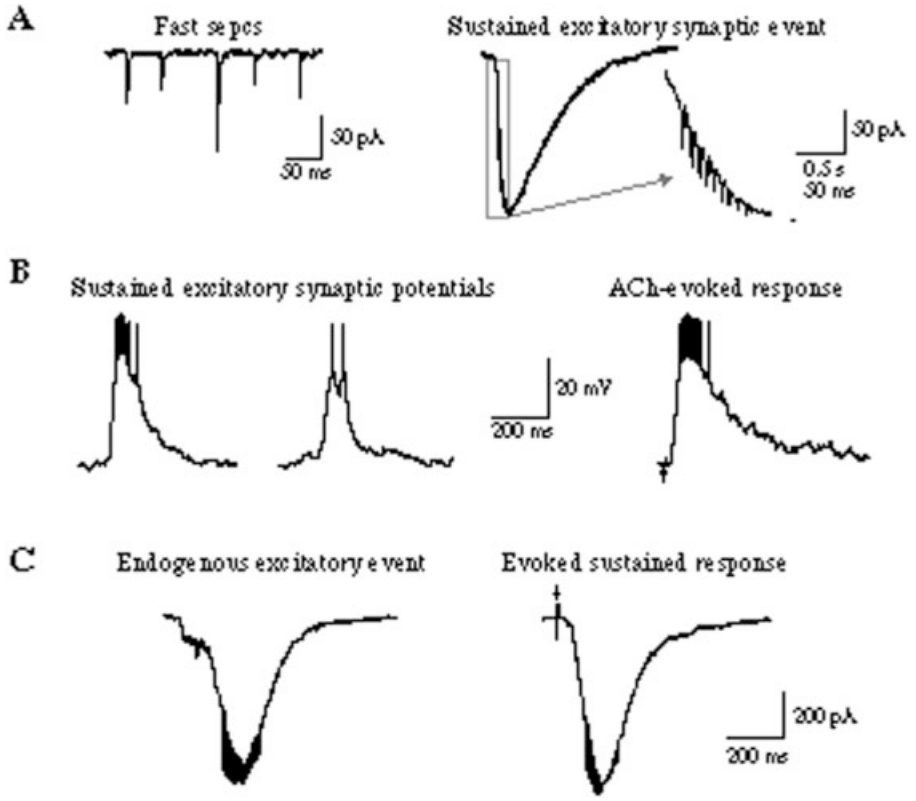


**Figure 6** Endogenous and evoked synaptic activity in motor neurons in the *Drosophila* larval CNS. (A) Left: fast sEPSCs are present in nearly all recordings from neurons in the ventral nerve cord of mature larvae. Right: an endogenous, sustained (~1.5 s) excitatory current recorded in voltage-clamp (−60 mV); inset is shown with 10×-expanded time scale. As in the embryo, rhythmically occurring cholinergic presynaptic input drives excitatory responses and bursts of action potential firing in the postsynaptic motor neuron. (B) Left: endogenous excitatory synaptic potentials and action potentials recorded in current clamp. Right: excitatory potential evoked by 1-ms ACh application (arrow) is indistinguishable from endogenous synaptic potentials. (C) Endogenous sustained excitatory event (left trace) and a sustained synaptic response elicited by electrical stimulation (right trace, arrow) of the CNS in the same neuron. Both endogenous and evoked activity exhibit identical cholinergic dependence.

## Current and Future Directions: From Embryonic Transmission to Adult Synaptic Plasticity Studies

Recent technical advances and newly developed experimental preparations now make it possible to address the synapse-specificity of neurotransmission and central plasticity mechanisms in mutant backgrounds that previously could be studied only at the peripheral NMJ. One obvious area for study will be pathways specifically required for cholinergic transmission, such as the genes regulating presynaptic ACh synthesis and vesicular transport (D. Lee and D.K. O'Dowd, in preparation), and postsynaptic AChR expression and localization. However, many additional processes will also benefit from comparison of central and peripheral *Drosophila* synapses. For example, a recently identified novel synaptic mutant, *bad reception* (*brec*), displays a complete absence of glutamate receptors at the NMJ (D.E. Featherstone and K. Broadie, unpublished data). Does *brec* have a specific role in postsynaptic development/regulation of glutamatergic synapses, or is it more generally involved in postsynaptic mechanisms at multiple classes of chemical synapses? To test these possibilities, neuronal ACh responses and endogenous cholinergic activity have been assayed in CNS neurons in the *brec* mutant. Mutant neurons respond normally to ACh application, showing normal functional AChR expression. However, the percentage of neurons exhibiting endogenous excitatory activity, as well as the frequency of endogenous events in active neurons, are both reduced (J. Rohrbough and K. Broadie, unpublished

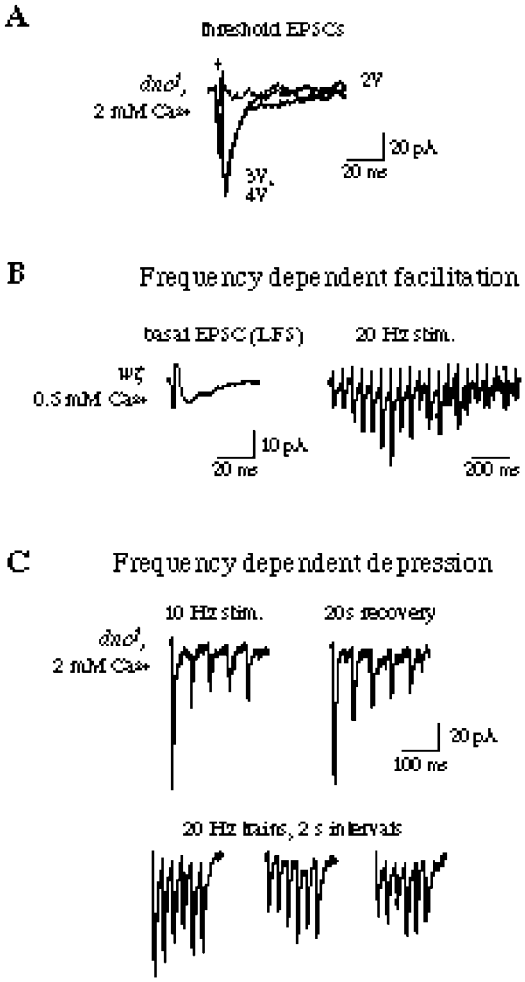


**Figure 7** Fast evoked excitatory synaptic transmission and activity-dependent plasticity in the larval CNS. (A) All-or-nothing fast excitatory synaptic currents (EPSCs) evoked by focal electrical stimulation (2, 3, and 4 volts) of the central neuropil adjacent to the motor neuron dendritic region [see Fig. 1(C) for example of stimulation configuration]. Recorded from a *dunce* mutant larval motor neuron in physiologic 2 mM $Ca^{2+}$ saline. (B) EPSC evoked by low-frequency stimulation (LFS; left trace) in 0.5 mM $Ca^{2+}$ saline in a wild-type larval neuron. EPSC amplitude rapidly facilitates and then declines in response to 20 Hz stimulation (right trace). (C) In physiologic 2 mM $Ca^{2+}$ saline, low-frequency stimulation evokes a strong EPSC in a *dunce* mutant larval neuron. Higher frequency (10 Hz) stimulation leads to rapid short-term depression of EPSC amplitude (top left trace), which fully recovers after 20 s (top right trace). In different neurons, 20-Hz stimuli appears to support both depression and facilitation (bottom traces); note that depression during the 20 Hz train is less pronounced than that during a 10-Hz train. Successive 20-Hz trains delivered at 2-s intervals reveals facilitation during high-frequency stimulation (lower middle and lower right trace).

data). These data suggest that *brec* function is most likely selective to glutamatergic synapses, and that glutamatergic neurotransmission in the CNS may regulate the frequency of the rhythmic currents in motor neurons that drive peristaltic locomotion (Cattaert and Birman, 2001). Studies of this nature promise to greatly increase our understanding of synapse-specific mechanisms in *Drosophila*.

Work in the embryonic and larval CNS to date has focused on several areas: techniques to reliably identify neurons or neuronal subpopulations; characterization of functional electrical and endogenous synaptic transmission properties in these neurons; and protocols to reliably elicit synaptic transmission to assay fundamental evoked transmission properties. An important further step is to develop and extend these approaches toward assaying forms of functional synaptic plasticity most relevant to behavioral modulation. Specifically needed are investigations of both short-term and long-term activity-dependent alterations in central cholinergic transmission, and established stimulation paradigms which effectively reveal common forms of central transmission modulation, including paired-pulse and short-term facilitation, and posttetanic potentiation. Likewise, it is important to determine whether frequency-dependent synaptic depression is an important component of plasticity in *Drosophila* circuits. Preliminary studies in the larval CNS reveal that cholinergic synaptic inputs onto motor neurons exhibit both frequency-dependent short-term facilitation and depression in different experimental conditions [Fig. 6(B) and (C); J. Rohrbough and K. Broadie, unpublished data]. A major objective is to determine whether long-term potentiation (LTP) and/or long-term depression (LTD) are active at these central *Drosophila* synapses, as has been suggested in other insects (Oleskevich et al., 1997), and what cellular pathways, such as such as the $Ca^{2+}$- and cAMP-dependent signaling, are involved.

Although progress is being made toward these goals, a sizeable gulf remains between these recent cellular assays in the embryonic and larval CNS, and successful application of similar approaches to adult CNS areas. One key target is the *Drosophila* mushroom body (MB), a paired L-shaped structure of ~80 $\mu$m dimensions containing ~2500 neurons per hemisphere, which is extensively implicated in olfactory learning and memory behavior. The MBs and their role in behavior have received renewed and intense attention recently (Armstrong et al., 1998; Pascual and Preat, 2001; Waddell and Quinn, 2001; recent reviews by Davis, 2001; Roman and Davis, 2001; T. Tully, 2002, this issue). Extracellular field recordings of evoked population responses in the (much larger)

MBs of honeybees have recently demonstrated both cholinergic synaptic transmission (Oleskevich, 1999), and long-term synaptic plasticity (Oleskevich et al., 1997) similar in many aspects to that recorded in mammalian hippocampal slices. Synaptic function underlying behavioral plasticity in the MBs and central brain of *Drosophila*, however, has so far been only indirectly addressed. Conditionally disrupting synaptic function throughout the MBs with the temperature-sensitive *shibire* protein has demonstrated a requirement for MB synaptic transmission in memory retrieval (Dubnau et al., 2001; McGuire et al., 2001). In the last year, $Ca^{2+}$ imaging experiments have provided the first physiologic view of odorant-induced MB activity at the cellular level (Waddell et al., 2000; Wang et al., 2001), and have shown altered levels of $Ca^{2+}$ activity in known learning and memory mutants (Rosay et al., 2001; reviewed by Carlson, 2001; Davis, 2001). To date, such optical methods represent the practical limit to physiologic access in the *Drosophila* MB.

Although certainly speculative at this time, it is nevertheless tempting to envisage functional synaptic recordings and activity dependent plasticity assays in normal and mutant *Drosophila* brains in the near future. A whole-brain recording preparation? Brain slice preparations? An increasing motivation to adapt these approaches to *Drosophila* is clearly evident. Developing the resources to carry out detailed cellular assays is obviously necessary to maintain and further exploit *Drosophila* at the forefront of experimental models being used to unravel the genetic and molecular bases of behavior.

In conclusion, the recent advances made in the emerging functional studies of the *Drosophila* CNS are both exciting and gratifying. Preliminary reports suggest that mechanisms underlying expression of functional neuronal properties and synaptic transmission in *Drosophila*, like those that control cell fate, axon guidance and synapse formation, are highly conserved across species. Because of this, the exploitation of the powerful molecular genetics in this model system offers the prospect of rapidly identifying and understanding the regulatory mechanisms orchestrating the functional development of neuronal circuits, and provides a means for the investigation of the cellular mechanisms of neuronal plasticity that underlie behavior.

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# THE PERSISTENT VEGETATIVE STATE

## *NEUROETHICS, COMMUNICATION AND EVIDENCE-BASED CLINICAL DECISION-MAKING*

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### Introduction:

Jennett and Plum[1] first described the persistent vegetative state in 1972 as a *syndrome in search of a name*. At this time *the persistent vegetative state* (PVS) designation remains unchanged, and describes a tragic condition in which vegetative functions are retained in severely brain-damaged patients who have no awareness or cognitive function. These vegetative functions include sleep-wake cycles, autonomic control, and respiratory drive.

In 1994, the Multi-Society Task Force [2,3] characterized patients in the PVS as follows:
There is no evidence of awareness of self or environment and an inability to interact with others; no evidence of sustained, reproducible, purposeful, or voluntary behavioral responses to visual, auditory, tactile, or noxious stimuli; there is no evidence of language comprehension or expression; periods of intermittent wakefulness manifested by the presence of sleep-wake cycles; sufficiently preserved hypothalamic and brainstem autonomic functions to permit survival with medical and nursing care; bowel and bladder incontinence; and variably preserved cranial nerve reflexes. In brief the patient in the PVS has no sustained voluntary or purposeful behavior of any kind.

Although in widespread use, the term *persistent vegetative state* (PVS) is being replaced by the term *vegetative state* (VS) which refers to both the persistent vegetative state (PVS) and the permanent vegetative state (which had also been designated PVS, which had further confused the issue)[4].

Roving eye movements and grimacing and non-purposeful motor movements are characteristic of many patients in the PVS. While some may object to use of the term *vegetative* to describe this condition, it remains a clinical descriptor of the clinical-pathological entity of the PVS. The term eyes-open coma has also been used to describe such patients.

### Differential Diagnosis and Clinical Decision-Making:

The differential diagnosis of PVS includes coma; the minimally conscious state[5]; akinetic mutism and the locked-in syndrome[2,3]. Patients with severe closed head injuries or severe hypoxic-ischemic injury resulting in the PVS can be maintained for many years with nutritional and hydration support, along with fastidious care-giving to prevent pressure ulcers and other complications of prolonged immobility. Bioethical issues related to these patients including autonomy and loss of personhood are key factors influencing clinical decision-making, yet the individual patient's wishes may not be known. Indeed, the most highly profiled patients in the public arena who were in the PVS (Karen Ann Quinlan, Nancy Cruzan and Terri Schiavo) left no clear indication of their personal preferences, and were associated with lengthy and contentious legal battles.

It is important to note that the PVS and minimally conscious state are clinical diagnoses, and not neuropathologic. However, the neuropathologic basis for the PVS has been described, although neuropathological findings for the PVS patient may differ from patient to patient. As indicated by Thogmartin[6], the interval between brain injury and death affects the nature and severity of pathologic changes. However, two major patterns have characterized most reports relating to the neuropathology of the PVS due to either acute traumatic or non-traumatic brain injury:

diffuse axonal injury (associated with shearing injury after acute trauma), and diffuse laminar cortical necrosis (due to acute global hypoxia and ischemia).

**Demographics:**

An estimated 10,000-25,000 adult patients and 4,000-10,000 children are in PVS in the U.S[2,3]. Most patients are cared for in long term care facilities and supported with artificial hydration and nutrition. The challenging and sometimes contentious issues related to confirming the neurological diagnosis, along with medical-ethical, legal issues related to PVS have been the subject of national and international debate as recently illustrated by the tragedy of Terry Schiavo [7-10]. The Multi-Society Task Force in 1994 summarized guidelines for the diagnosis and prognosis of the PVS which provide a valuable framework for neurologists to develop recommendations and to assist family decision-making. Most patients in the PVS survive an average of two to three years, but with documented long-term survivors of ten or more years.

**A Patient in the Persistent Vegetative State:**

A 52 year-old man suffered prolonged cardiac arrest, and was resuscitated by EMS personnel following 10 minutes of having no pulse per bystander evaluation. The patient initially required ventilator support, and experienced prolonged generalized tonic-clonic seizure activity and myoclonus which was eventually controlled. Two weeks following the acute event, he developed roving eye movements with occasional spontaneous movements of his extremities. He was successfully weaned from the respirator. The patient remained unresponsive to pain and verbal stimuli. Reflex non-purposeful limb movements were occasionally noted. The EEG was diffusely slow, and demonstrated changes indicative of physiological sleep-wake cycles.

A PEG tube was placed three weeks after the initial event at the recommendation of the primary care physician in consultation with the patient's wife. The patient subsequently maintained a regular pulse, respirations and blood pressure. A diagnosis of PVS was made by a consulting neurologist 3 months post-arrest, and was confirmed by a second neurologist. No change in the patient's overall neurological status was noted after 6 months in this condition while he was maintained in a nursing home. A DNR order was provided by the patient's wife who was his designated surrogate decision-maker. The patient did not have an advance directive, and had stated on several occasions while in good health that in the event of serious, irreversible illness, "My family will know what to do." However, he had refused to discuss his own preferences and avoidances should he ever lose decision-making capacity. Seven months post-arrest and while in the nursing home, he developed generalized sepsis secondary to urinary tract infection associated with resistant organisms.

Comment: The Multi-Society Task Force on PVS[2,3] has indicated that patients with PVS secondary to hypoxic-ischemic injury are unlikely to recover meaningful neurological function if no recovery has occurred within 60-90 days following the acute injury. Although patients in the PVS frequently demonstrate eye-opening and non-purposeful motor activity, they have no awareness of their surroundings or cognitive ability.

**Critical Questions:**

Numerous questions may arise in the context of establishing a diagnosis of the PVS. They will often be instrumental in establishing the care plan and in subsequent clinical decision-making. A number of key questions are listed, and include those provided by Hook and Mueller [9].

> A critical question to ask the family or other designated surrogate decision-maker: **"What is your understanding of your (father, mother, sibling's...) condition?"**
>
> It is not always warranted to assume that family members will have a clear understanding of the essentially permanent and hopeless condition which the patient in the PVS represents. It is usually helpful to ask if there

are specific questions regarding prognosis, as well as medical and nursing support required for long-term care of the patient.

**Additional key questions:**

- Who should make the final decision regarding life support? What is the most effective way for the neurologist to discuss this issue with the family decision-maker so as not to risk guilt for the family member.
- Is a feeding tube mandatory for comfort care, or is it a medical intervention that can be refused, withheld or withdrawn?
- How do you know whether the patient is in the PVS versus the minimally conscious state?
- Is there any hope of cognitive and other neurological recovery? What is the likelihood of meaningful neurological recovery even after one year in the PVS?
- What were the patient's expressed wishes, or did he/she ever indicate what those preferences might be if he were in these circumstances?
- Who should speak for the patient when he cannot do so?
- Is removing a feeding tube painful?—is this considered euthanasia?
- Doesn't the patient experience pain from starvation if a feeding tube is removed?
- Is it ethical and legal to withdraw or withhold life-sustaining medical interventions?
- Is terminal dehydration painful?

**Myths and Misinformation:**

Major myths persist regarding the public's perception that withdrawal of a feeding tube is associated with pain even for the patient in the PVS. It is important for the neurologist to ask the patient's family to describe their understanding of the PVS and implications for prognosis in order to guide decision-making. It should also be noted that decision-making related to withholding or withdrawing care in patients in PVS and other end-stage illnesses may be greatly influenced by culture and ethnicity. Decisions cannot always be resolved on the basis of poor prognosis alone, as noted by Topper and Nacimiento [10]. Decision-making in other hopeless situations is also strongly influenced by ethnic and cultural background Crawley et al[12], Irish et al [13], Jenkins et al[14]. Most experienced neurologists have also experienced the family member from "out-of-town" who appears as a late arrival to the scene of a hopelessly and irreversibly brain-damaged patient, and insists that "everything be done." This often occurs even when the majority of local family members have decided to avoid heroic interventions and to limit life-prolonging maneuvers.

Three broad categories of brain injury are responsible for PVS: 1) Acute brain injury, traumatic and non-traumatic; 2) neurodegenerative and metabolic disorders; and 3) developmental malformations. These have also been recently discussed in C*ontinuum*[15] .
Acute brain trauma with associated diffuse axonal injury represents the most common cause of PVS. Severe hypoxic-ischemic brain injury such as occurring after prolonged cardio-respiratory arrest may result in PVS. Severe end-stage brain disease occurring in Alzheimer's disease and Creutzfeldt-Jacob disease may result in PVS, although there is controversy regarding these last two entities and their association with the PVS. Pediatric conditions such as

ganglioside storage disease and ceroid lipofuscinosis, as well as anencephaly and hydranencephaly progress to the PVS.

Kinney and Samuels (1994)[16] have reviewed the neuropathology of the PVS. Loss of awareness appears to result from widespread and bilateral damage to the cerebral cortex, subcortical connections of the cerebral hemisphere white matter and the thalamus. Kinney et.al.[16] emphasized the special importance of the thalamus being responsible for cognition and awareness based on neuropathological findings of Karen Ann Quinlan. More recently the neuropathology report of Terri Schiavo has supported this notion[6]. There are no completely reliable prognostic indicators for patients in the PVS, but guidelines exist in part reflecting the underlying etiology of the PVS in individual patients.

There is lack of correlation between CT brain imaging and the ability to predict development of the PVS. Diffusion weighted MRI imaging offers the possibility of indicating the extent of neuronal injury resulting from global cerebral hypoxic-ischemic insult. Metabolic changes in the brains of patients in PVS have also been investigated with positron emission tomography (PET). Rudolph[18] et al demonstrated that severe reduction of radio-labeled benzodiazepine brain receptor ligand binding correlated with poor outcome in patients in PVS.

**Communicating with the family of the PVS patient.**

***The single biggest problem in communication is the illusion that it has taken place.***

.

George Bernard Shaw

Because we are all in contact with each other, we may assume that we have been adequately communicating. Hallenbeck[19] has indicated that as we assume that our natural abilities are by themselves sufficient. With palliative care, this may not always be the case. Good intentions alone may be inadequate to meet the great challenges inherent in providing the highest quality palliative care and support for the patient's family. There is overwhelming evidence that clinicians have difficulty communicating well with patients and families, in general. Stakes are high, and decisions often agonizingly difficult. Taboos, mistaken assumptions, obscure communication and comprehension stand to complicate the goals of even the best intentioned physician. Misunderstanding and misinterpretations frequently occur, and the details of previous discussions may be soon forgotten by a distressed family member.

Palliative care literature has also documented poor communication by physicians.
Tulsky [20] reported that resident physicians dominated allotted speaking time, and in only 10 percent of cases were the patient's values and care plan goals discussed. Several initiatives are underway to enhance communication skills for physicians involved in the care of seriously ill or dying patients and their families [21].

Hallenbeck[19] has summarized several features of communication which occur between physicians and patients and their families:

- Physicians (and other clinicians) often talk too much and do not listen enough.
- Physicians tend to focus exclusively on the cognitive (thinking) aspects of communication and ignore affective (emotional) aspects.
- Physicians tend to force their agendas over patient and family agendas.

Identifying the mood of family members and dealing with emotions is helpful as a means to successfully communicate prognosis and to facilitate decision-making. Empathic communication of bad news should be a goal of all practitioners, including discussion of poor prognosis with family members of the patient in the PVS.

Some important questions and issues to consider include the following:

**Key question: "What is your understanding of your mother condition?"**

This is probably the most important question to ask a family member. It is probably better not to assume that the patient's family understands, or has even been told in any detail about the broad implications of the PVS. There is probably even greater likelihood of confused messages in large academic medical centers where rotating attending and resident schedules sometimes makes it less than clear who is actually in charge of the patient's care, and who will guide important medical decision-making. A change in attendings may also be associated with a very different approach to the care plan and overall decision-making.

**"She is following me with her eyes—so she must be able to see me."**

The television news re-plays of Terri Schiavo was unnerving to many members of the American public, and could have conveyed the mistaken impression that the eyes-open state of this unfortunate patient was associated with vision and therefore awareness. In actuality, the post-mortem examination of Terry Schiavo indicated destruction of the occipital cortex, precluding any possibility of an intact visual system or ability of any patient to see.

**What about the term *vegetative*?**

The term *vegetative* was first used by Jennett and Plum[1] in their landmark paper first describing the vegetative state in search of a name. It is this writer's experience that some health professionals are uncomfortable with the term *vegetative* because of its implications comparing the patient to a vegetable. The expression, "I don't want to be a vegetable" expresses a strong sentiment shared by many Americans with reference to prolonged life support in the absence of awareness and meaningful existence.

**Some myths and misconceptions:**

"My family will know what to do."

Most experienced physicians can attest to the fact that many/most families are not prepared to make life-and-death decisions on behalf of an incapacitated patient unless prior discussion or written advance directive has been completed. Yet many patients and health individuals are reluctant to have these critical discussions with their families or other potential surrogate decision-makers.

**"I would treat my dog better than that."**

Quote from radio talk show call-in listener to neurologist guest during debates surrounding decision-making for Terrl Schiavo when her feeding tube had just been removed.

Frederich has summarized the role of hydration and nutrition for the terminally ill patient in an American Academy of Hospice and Palliative Care bulletin (2002)[22]. Dunn (1994)[23] has produced a very useful and easy-to-read monograph for family caregiver regarding decision-making regarding CPR, artificial feeding and other key decisions.

**Often unhelpful:** Asking a family, "What do you want us to do?" Instead, offer your professional opinion. Ideally, you have listened to family members or other decision-makers, and have shared important information regarding issues requiring specific decisions. These might include CPR and request for DNR orders; whether or not

to treat life-threatening infections; use of continued nutritional support via feeding tube; and hydration. While families usually welcome your involving them in decision-making, they frequently are quietly seeking direction from you as the expert neurologist. It is appropriate for you to offer your opinion and recommendations based on medical evidence. For example, long-term prognosis of patients diagnosed with the PVS can be the basis for discussing to inadvisability of providing futile care in a setting where likelihood of neurological recovery is virtually non-existent.

It is always important to document in the medical record a summary of discussions with family or other surrogate decision-makers. This should include a summary of the participants involved in the discussion; summary of the essence of the discussion, including focus on benefits and burdens of any specific intervention, such as blood-drawing for electrolyte testing, etc.; use of feeding tube; future use of antibiotics for life-threatening infections.

**Communication in general varies by personal experience, ethnicity and culture.** –*One size does not fit all.* In general, how might you respond to the following comments from a patient or family member who has a serious, life-limiting illness?

"I don't want to talk about it."

"Don't tell her that she has a terminal illness…"

"It's in God's hands—do everything possible." –reflecting the over-riding sanctity of life and sometimes mis-trust of the medical establishment (African-Americans)

"Talk to my son—he can make the decisions." Family decision-making, and different views of patient autonomy, i.e., family decision-making rather than individual (Hispanic patient; far-Eastern cultures).

"Presentismo"—Hispanic—live life for the moment, avoid advance care planning, etc.

**Family discord and dissension**

Who is speaking for the patient, and does the family speak with one voice?" If a large number of family members appears for a family conference, and is in disagreement, who will speak on behalf of the family? It is helpful to identify one family spokesperson who speaks on behalf of the entire family if possible, to avoid attempts at chaotic and disjointed decision-making.

Active communication with family members of the PVS patient is crucial9. A basic medical knowledge of the dire circumstances of the patient in PVS must be conveyed to the family. It is usually worthwhile to ask the family to describe their understanding of the family member's condition, including prognosis. Great sensitivity must be provided in discussions with the family, in allowing for the scope of the family tragedy. Prognosis is difficult to estimate during the early phases of the vegetative state, but can be made with greater confidence when following the Multi-Society Task Force guidelines. Although a majority of randomly surveyed Americans have indicated no desire to be supported with artificial hydration and nutrition if they were to be in PVS, decision-making on an individual patient can be challenging if there is no completed advance directive to guide decision-making.

**Medical Futility and Utilization of Consulting Resources:**

Medical futility *means different things to different people*. Authors have attempted to define parameters of futile care (Kasman, 2004[24]), as well as the relationship of DNR orders to medical futility Cantor et al, 2003[25]). The Ethics Committee, Pastoral Care Service and Palliative Care Service in the hospital environment can be helpful in addressing and sometimes resolving difficult issues related to providing futile care.

**When is care futile, and who decides?**

Decision-making with regard to discontinuing or continuing life support for a patient diagnosed with the PVS should be based on ethical principles. The concept of patient autonomy may come into conflict with medical futility as defined by professional medical caregivers. Decisions made on a case-by-case basis may sometimes risk appearing arbitrary. A call for a more principled approach based on ethical and considerations has been recommended. Decision making should strive to avoid *paternalism,* a mode of medical care which has long been discredited.

**Palliative Care**

Palliative Care has been defined by The World Health Organization as the active total care of patients whose disease is not responsive to curative treatment . Control of pain, other symptoms, and of psychologic, social, and spiritual problems are of paramount importance for any patient with severe life-limiting disease. While some goals related to the conscious patient (i.e., psychologic, social) do not necessarily apply to the patient in the PVS because of absence of awareness, the over-arching goal of palliative care is the achievement of the best possible quality of life for patients and their families. Many aspects of palliative care are also applicable early in the course of the illness in conjunction with disease modifying treatment. While all hospice care is palliative care, all palliative care is not hospice.

Preserving the overall dignity of the patient in the PVS by attending to oral hygiene, incontinence care, and turning to prevent decubitus ulcers are of paramount importance. Treatment of life-threatening infections, and withholding/withdrawing of other medical interventions, such as feeding tubes, etc. can be emotional issues for many families. Decisions will vary greatly on a case-by-case basis.

**Palliative care:**

- Affirms life and regards dying as a normal process
- Neither hastens nor postpones death
- Provides relief from pain and other distressing symptoms
- Integrates the psychological and spiritual aspects of patient care
- Offers a support system to help patients live as actively as possible until death
- Offers a support system to help the family cope during the patient's illness and in their own bereavement.

**Hospice**

Modern hospice began in London, England during the late 1960's, and was instituted in the United States as the Hospice Medicare benefit in 1982. Approximately one-third of patients dying of a terminal illness in the U.S. receive hospice benefits. The referring physician must estimate that the patient has six months or less of life expectancy, and that the primary goals of care are focused on providing comfort care[26]. Neither life-prolonging nor life-shortening interventions are part of the hospice plan of care.

The hospice interdisciplinary team consists of hospice nurses, social workers, chaplains, and medical director. The referring physician is encouraged to participate in the care plan and medical decision-making, but may choose to convey care of the patient entirely to the hospice team. Approximately 50% of hospice patients have a diagnosis of cancer or other terminal malignancy, while the remainder of hospice patients has congestive heart failure, COPD, or another severe life-limited illnesses. These include end-stage neurological disease. The patient with PVS would qualify for hospice care if the overall philosophy of hospice care was accepted by family decision-makers.

**Conclusions:**

As Angell[27] has noted, it is remarkable how rapidly the concept of brain death attracted an ethical, social and legal consensus along with medical endorsement. There is no longer any requirement to continue cardiopulmonary

resuscitations for a patient who is diagnosed with brain death. However, this landmark approach did not address the issue of patients in the PVS. These patients have retained brainstem and hypothalamic function required for prolonged survival, and such patients may survive for years when provided artificial nutrition and medical support.

Following the landmark case of Karen Quinlan in 1975 family members were provided a clear right to discontinue life support for patients who were in the PVS. Family decisions may involve a request to continue support for patients in PVS even over the objection of physician, hospitals, and other care givers. The case of Helga Wanglie as indicated by Angell provides such an example. Courts in Minnesota upheld Mr.Wanglie's continued insistence that his wife who was in the PVS be continued on life support.

An approach suggested by Angell[27] would presume that patients in the PVS would not want to be kept alive indefinitely, which she suggests is a presumption supported by public opinion polls. Presently it is assumed that patients in the PVS would want to be kept alive, unless an advance directive or other clear statement to the contrary was evident. This and other issues related to the patient in the PVS will provide a focus of further discussion in the years ahead [28-32].

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# Medical School Application Tips

## Pre-requisite Courses

The minimum science course work necessary for preparing for medical school and the MCAT includes two semesters each of general biology, general chemistry, organic chemistry, and physics. These courses should be completed no later than the end of your junior year so that you will be prepared for the MCAT exam. The MCAT is now offered 22 times each year which allows students more flexibility as they prepare to apply to medical school.

*Medical school admission's committees focus attention on overall GPA, Science GPA, MCAT scores and exposure to the clinical environment achieved through volunteer work, internships, shadowing or employment.*

Each medical school determines its specific requirements for admissions. These requirements can be found in the Medical School Admissions Requirements (MSAR), an official guide published by the Association of American Medical Colleges. Additional recommended electives for medical school are Biochemistry, Molecular Biology, Microbiology, courses that expose students to Multi-cultural Diversity, Biomedical Ethics and Medical Terminology.

## Medical College Admission Test (MCAT) and The Application Process

The MCAT is required by allopathic,osteopathic and podiatric medical schools. This standardized test is directed at an introductory level of knowledge for science courses and comprehensive skills in reading and writing. To do well on these tests students must have analytic skills and problem solving ability. Adequate preparation for these tests is essential. A student should aim to take the test only once. Although some professional schools will consider only the highest score or the most recent score, other schools will average all test scores. To adequately prepare for these tests, students must not only be knowledgeable about content, but must also be familiar with the test format and develop the resilience and stamina needed to concentrate for these marathon length exams. Most standardized tests have a deadline for applying that is about 6 weeks prior to the scheduled test date. Students must allow another 6 to 8 week for the test scores to be forwarded to the professional schools when determining when to take the test and apply to professional school.

The MCAT is offered only in a computerized format and is offered 22 times during the year. Applicants must register for this exam on line at their local Prometrics Testing Center. The verbal reasoning, biological reasoning and physical science sections are graded out of 15 points and the writing section is graded on a letter scale from J to T. The highest total score one can achieve on the MCAT is a 45; the national average is currently at 30.

## Application Service (AMCAS)

Centralized application services are used to apply to most allopathic, osteopathic, and podiatric programs. Students who apply to any of the professional programs submit just one application through the service. The centralized service verifies the information provided on the application and submits the application to the professional schools designated by the applicant. The verification process may take up to 6 weeks and this

time should be factored in when trying to meet application deadlines. Most professional schools require additional information – called a secondary or supplemental application – from the applicant once they receive the student's application from the centralized service.

**Design a Plan!**

**FIRST YEAR**

***Fall Semester***:
• Explore various majors and declare as early as possible. Because you will need to complete the 32 credit hours of sciences before you can take the MCAT it will be necessary to get your science courses underway as quickly as possible.
• Make an appointment with your advisor to discuss professional goals and determine an academic game plan to ensure you will have taken all courses needed to prepare for standardized admissions tests in your field of interest by the time you take the exam (usually at the end of your junior year).
• As soon as you get settled into your classes, contact the Pre-professional Health Advisor, to make an advising appointment to discuss professional goals and discuss what the professional will want you to have completed by the time you're ready to apply.

***Spring Semester***:
• Meet with your academic advisor to discuss your progress.
• Search for shadowing and volunteer opportunities in your field of interest.
• Explore areas of community outreach to establish your humanitarian interests.

***First Summer:***
• Shadowing, working, or volunteering to gain insight into your career choice.

**SECOND YEAR**

- Fall Semester: Meet with your advisor to discuss your Spring schedule.
- Learn about the MCAT
- Continue volunteer work in your field to whatever degree manageable during the academic year (Grades should always take priority)

***Spring Semester:***
- Discuss your academic progress with your advisor and adjust your academic plan, as needed. Meet with Pre-Health Professions Advisor to make review your progress and the next phase of preparation
- Explore career options and alternatives, if your GPA is not adequate or your career interests have changed.
- Sign up to take a free practice MCAT through Kaplan, if available

***Second Summer***:
- Work/volunteer to gain insight of your career choice.
- Explore available internship and research opportunities
- Get involved in the community

## THIRD YEAR

*Fall Semester:*

- Meet with your advisor to discuss spring schedule.
- Get organized, order review booklets and practice tests to prepare for standardized exams.
- Explore various professional schools and determine to which ones you will apply.

*Spring Semester:*

- Discuss your academic progress with your advisor. Determine if your GPA is competitive and whether or not this is the year you should apply to professional school.
- Establish a file with the College's Health Professions Committee, if available at your institution.
- Start worksheets for on-line centralized application services and/or request applications from schools that do not participate in the centralized application process. (Online AMCAS application is available early in May of each year)
- Collect materials needed to fill in application and start working on application essay.
- Apply and study for standardized admissions tests. Check the deadlines and do not miss them.
- Practice, practice, practice taking the standardized admissions test.
- Take the standardized admissions test and request that scores be released to your college or university, schools to which you are applying and the application service (if appropriate).
- Request letters of evaluation from faculty and health professionals who know you well.

*Third Summer:*

- Continue to work/volunteer in your field of interest and community outreach projects.
- Complete your applications and submit early. Early decision program applications must be submitted to the schools by August 1st.
- Retake standardized admissions tests, if necessary.

## FOURTH YEAR

*Fall Semester:*

- Meet with your advisor and prepare for graduation.
- Respond promptly to requests for secondary applications from each professional school.
- Prepare for interviews.
- Interview and wait.
- Search for sources of financial aid.

*Spring Semester:*

- Send updated transcripts directly to the professional schools to which you have applied.
- Wait for decisions. Be sure to let your institution know the final outcome.
- Discuss alternatives with your advisor.

# Research Fellowships for Summer Research, Graduate School, and Medical School

One of the most important skills you can learn as a developing scientist is how to convince other people that your research questions are really interesting. So interesting in fact, that they will give you money to pursue the answers to those questions. If you are lucky you will spend the rest of your life trying to answer them. I am not sure you ever perfect the art of grant writing, but practice makes almost perfect. And there is no time like the present to start working on it. Over time, you will develop a track record of applying for and, hopefully, attaining funding for your work. One more thing, this looks great on your resume as you begin looking for that future position as an independent researcher. So let's look at the options…

## Undergraduate Science and Engineering Scholarships

Goldwater Science Scholars (Rising Juniors and Seniors): http://www.act.org/goldwater/

- Provides up to $7500 dollars towards tuition, room and board, books etc. Renewable for a second year if a junior. ~300 awarded each year.

NIH Undergraduate Scholarship Program: http://ugsp.info.nih.gov/

- The program offers competitive scholarships to students from disadvantaged backgrounds who are committed to careers in biomedical, behavioral, and social science health-related research. The program offers scholarship support ($20,000), paid summer research training at NIH, and employment/training after graduation.

## Summer Undergraduate Research Experiences

These are full-time summer research programs that typically provide a stipend (~$3500), travel funds and housing at the research site. Some institutions provide summer fellowships for their own students to continue working on their research during the summer. Other schools run nationally competitive summer research programs. Several undergraduate institutions keep up to date lists of these programs. Two good sites are:

http://www.psych.westminster.edu/psybio/internops.htm
http://www.swarthmore.edu/Admin/health_sciences/summer_opportunities.html

National Science Foundation Research Experiences for Undergraduates

- You can search for experiences in particular scientific fields or for programs at particular institutions at the NSF website: http://www.nsf.gov/crssprgm/reu/reu_search.cfm

National Institutes of Health Summer Internship Program in Biomedical Research (SIPS)

- This is a 10 week summer program at the NIH: http://www.training.nih.gov/student/sip/index.asp

Application deadlines are generally between January and March for the following summer.

**Still want to spend some time doing research before committing to graduate school? The NIH has a post-baccalaureate year-long research program that you might be interested in:**

NIH Post baccalaureate Intramural Research Training Award (IRTA):
http://www.training.nih.gov/student/Pre-IRTA/previewpostbac.asp?AppType=Postbac

**So you made it to graduate school…now it is time to apply for pre-doctoral fellowships:**

NSF Graduate Research Fellowship Program

- http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=6201
- The Graduate Research Fellowship provides three years of support for graduate study leading to research-based masters or doctoral degrees and is intended for students who are at the early stages of their graduate study. Apply during your senior year or in the first year of graduate study. ~1000 are awarded each year.

NIH Ruth L. Kirschstein National Research Service Award (an F31 grant)

- http://grants.nih.gov/training/nrsa.htm#F31
- This is the format for grant proposals that you will write as a post-doctoral fellow (F32) and principal investigator (R15 and R01). The F31 is the predoctoral award. There are separate awards for minority students and for students with disabilities. These grants are usually written in conjunction with your advisor once you have begun your thesis research. You request a certain period of funding (~2-3 years) based on the length of the project. There are also F30 awards for predoctoral students studying for an MD/PhD

Hertz Foundation Fellowships

- http://www.hertzfoundation.org/dx/Fellowships/
- The fellowship is a five year award to those pursuing a Ph.D. in "applied sciences" and consists of a $28,000/9-month personal stipend, full tuition equivalent, and is renewable for up to 5 years.

**Finally, if you work on a specific human disease or disorder there are usually private foundations that support the research. Often they will offer a limited number of pre- and post-doctoral fellowships.**

**But wait, I want to be a doctor or a dentist and I am still interested in research…**

Howard Hughes Medical Institute (HHMI) Research Training Fellowships for Medical Students

- http://www.hhmi.org/grants/individuals/medfellows.html
- The Medical Fellows Program supports a year of full-time biomedical research training for medical and dental students. Applicants must be enrolled in a U.S. medical school or dental school and the fellowship research may be conducted at any academic or nonprofit institution in the United States, except the National Institutes of Health. For the 2006 competition: An annual stipend of $25,000, an annual fellow's allowance of $5,500, and an annual research allowance of $5,500.

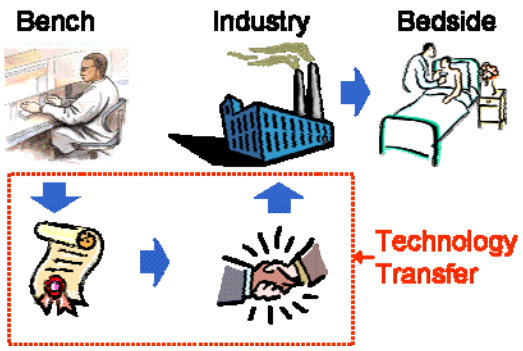
NIH Medical and Dental Student Research Programs:

- http://www.training.nih.gov/student/index.asp
- Look in the Medical/Dental Section of this website to learn about the different opportunities available at the NIH

# Careers in Technology Transfer

## What is technology transfer?

Technology transfer is the process of transferring scientific findings from the laboratory to entities that can develop them into products, which can ultimately benefit the public.




Technology transfer managers liaise with scientists who may have a commercially valuable idea, assess its commercial potential, manage the patenting process (if it is something which can be patented) and then try to ensure that the idea is exploited successfully, usually through licensing or sometimes by forming a spin-out company.

Technology management usually involves legal work such as licensing, and forming an extensive network of contacts with businesses seeking to license an invention or support a spin-out company based on the technology.

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**Links:**

### General Information about Technology Transfer

Professional organization for technology managers working in *academia*, AUTM: http://www.autm.net/

The professional organization for technology managers working in *industry*, LES: http://www.usa-canada.les.org/

Technology transfer for MUSC is managed by the MUSC Foundation for Research Development: http://frd.musc.edu/

### Internship opportunities in Technology Transfer

National Institutes of Health: http://www.ott.nih.gov/about_nih/intern.html

National Cancer Institute: http://ttc.nci.nih.gov/employment/

USCLA http://www.research.ucla.edu/oipa/interns/

Boston University http://www.bu.edu/otd/education/internships.html

### Education programs in Technology Transfer

To learn about technology transfer education programs, click on the “Education” tab on this website: http://ttic.nal.usda.gov/

### General information on alternative careers

http://sciencecareers.sciencemag.org/

http://chronicle.com/

### Book

“Alternative Careers in Science: Leaving the Ivory Tower” by Cynthia Robbins-Roth http://books.google.com/books?id=ge5oyXwW6UQC Chapter 15.

# Careers in Medical Writing

Christine Lauay, PhD

Scientific medical writers (those with a degree in medicine, science, pharmacy or related fields) might prepare regulatory documents (clinical trial reports, integrated summaries of efficacy and safety, investigator brochures etc.), physician speeches, posters, sales training manuals, policy documents, journal abstracts and articles, advertising copy for pharmaceuticals, internet content, medical education materials, pharmaceutical marketing and advertising.

Marketing medical writers (those with a degree in journalism or English) might prepare advertising copy, articles, internet content, magazine articles (popular or professional press), marketing materials, newsletters, patient education materials, public relations materials, training manuals, and perform editing and proofreading.

The two main pathways to a career in medical writing:

- Scientists who learn writing, and
- Writers who learn science.

In addition, courses or programs in medical writing are available (e.g., University of the Sciences in Philadelphia Masters in Biomedical Communications, University of Chicago Graham School of General Studies, Medical Writing and Editing). Medical writing associations offer certification programs (eg, American Medical Writers Association: AMWA) for medical writers. Also, taking courses in pharmacology, statistics, and life sciences is useful.

As with any career, there are pros and cons that go with a career in medical writing. Medical writing can be a lucrative career, and writers generally rate themselves as very satisfied with their career. There is great flexibility with full time, part time, remote, and freelance opportunities. Medical writers are often required to interpret data and prepare a document in a short time, must be prepared to have their work heavily edited, and should not expect to be a author when a manuscript is published.

Some qualities important in a medical writer include: ability to gather, analyze, and summarize large amounts of data, ability to express ideas succinctly, a background in science or medicine, ability to work within a team, and ability to communicate with many professionals from many functional disciplines.

<u>Tips for those interested in starting a career as a medical writer</u>

- Join AMWA or Drug Information Association (DIA).
- Read medical journals.
- Starting out can be tough – especially for regulatory writing. Try working first for a contract research organization (CRO) or small medical writing company as a freelance or part-timer, or get into pharmaceuticals via some other avenue (eg, clinical research) to gain exposure to the documents being used. If regulatory writing is not what you are interested in, then try starting in ad work or continuing medical education (CME). Start as an editor.
- Look at company websites. Find a company you're interested in, call up the human resources person and ask for an interview (you're likely to get it, even if no job openings exist at the moment), then go and talk with as many people as time allows. Be prepared with lots of questions when you go (e.g.,

what kind of projects do the writers do? Publications, regulatory docs? What would someone in an entry level position do? What kind of training is available?). Also be prepared to answer questions (eg, why are you interested in the company? Why do you want to be a writer?).

- Be enthusiastic.
- Publish your own research and write reviews if completing a thesis.
- Develop relationships with more experienced writers who may give you a start.
- Write! For example, take a technical or science writing course or program (for example, Jackson Laboratory has an undergrad intern program for science writing, see http://education.jax.org/science_writer.html )

Data from the 2007 AMWA Salary Survey

*Region of Primary Work*

(some areas have higher population of pharma companies)

| | |
|---|---|
| NY, PA, NJ, DE, Eastern Canada: | 30% |
| Pacific NW, CA, HI: | 15% |
| Southeast US, MD, DC: | 13% |
| Midwest: | 13% |
| MA, CT, RI, VT, NH, ME: | 9% |

*Highest Education Level*

| | |
|---|---|
| Associate's degree or below: | 2% |
| Bachelor's degree: | 33% |
| Master's degree: | 35% |
| Advanced degree: | 30% |

*Field of Highest Degree*

| | |
|---|---|
| Science | (40%) |
| Liberal Arts | (11%) |
| Journalism | (5%) |
| Pharmacy | (5%) |
| Medicine | (4%) |
| Communication | (4%) |
| Public Health | (3%) |
| Technical Writing | (3%) |
| Nursing | (2%) |
| Medical Writing | (1%) |
| Other | (25%) |

*Primary Employer*

| | |
|---|---|
| Pharmaceutical company | (25%) |
| Communication or advertising | (11%) |
| Biotech company | (9%) |
| University or medical School | (9%) |
| Contract research organization | (7%) |
| Health care organization | (7%) |
| Journal or publisher | (5%) |

| | |
|---|---|
| Research or education | (4%) |
| Association or professional soc | (4%) |
| Medical device company | (4%) |
| Other | (12%) |

Useful Websites:
Science Magazine: http://sciencecareers.sciencemag.org/career_development
(click on "more topics", then "alternative careers")
American Medical Writers Association: http://www.amwa.org/
National Association of Science Writers: http://www.nasw.org/
Drug Information Association: http://www.diahome.org/DIAHome/
US Food and Drug Administration: http://www.fda.gov/
(eg, search for 'guidance' to see outlines of content of regulatory documents)
Salary.com: http://www.salary.com/

Some factors that affect income:
Degree, location, size of company, experience
The following data are from salary.com, in the Chicago area (accessed Feb 2008)
Medical Writer I (BA/Master's, 0-2 yrs experience):

| Benefit | Median Amount | % of Total |
|---|---|---|
| Base Salary | $57,337 | 70.3% |
| Bonuses | $64 | 0.1% |
| Social Security | $4,391 | 5.4% |
| 401k / 403b | $3,559 | 4.4% |
| Disability | $918 | 1.1% |
| Healthcare | $5,328 | 6.5% |
| Pension | $2,411 | 3.0% |
| Time Off | $7,506 | 9.2% |
| Total | $81,515 | 100% |

Medical Writer II (BA/Masters & 2-5 years experience):

| Benefit | Median Amount | % of Total |
|---|---|---|
| Base Salary | $67,194 | 70.7% |
| Bonuses | $368 | 0.4% |
| Social Security | $5,168 | 5.4% |
| 401k / 403b | $4,189 | 4.4% |
| Disability | $1,081 | 1.1% |
| Healthcare | $5,328 | 5.6% |
| Pension | $2,838 | 3.0% |
| Time Off | $8,835 | 9.3% |

| | | |
|---|---|---|
| Total | $95,001 | 100 |

Medical Writer III (BA/Masters & 2-5 years experience):

| Benefit | Median Amount | % of Total |
|---|---|---|
| Base Salary | $78,460 | 70.8% |
| Bonuses | $979 | 0.9% |
| Social Security | $6,077 | 5.5% |
| 401k / 403b | $4,925 | 4.4% |
| Disability | $1,271 | 1.1% |
| Healthcare | $5,328 | 4.8% |
| Pension | $3,336 | 3.0% |
| Time Off | $10,388 | 9.4% |
| Total | $110,765 | 100% |

# Neurons in Action 2

***Tutorials and Simulations Using NEURON***

*John W. Moore and Ann E. Stuart*


Neurons in Action 2 is the second version of a unique CD-ROM-based learning tool that combines hyperlinked text with NEURON simulations of laboratory experiments in neurophysiology. Neurons in Action's moving graphs provide insight into nerve function that is simply not possible with conventional, static text and figure presentations. Students discover how changing parameters such as neuronal geometry, ion concentrations, ion channel densities, temperature or degree of myelination affects the generation of action potentials, synaptic potentials, and the spread or propagation of voltages within a neuron. For instructors, minimovies of NEURON simulations are provided for use in lectures.

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